5574-33-8

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VOLUME 48

October-December, 1941 (INCLUSIVE)

NEW YORK

Press of
THOMAS J. GRIFFITHS SONS, INC.
Utica, N. Y.

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Proceedings
of the
Society
for
Experimental Biology and Medicine

Vol. 48. October, 1941. No. 1.

13200 P

Interstitial Myocarditis in Mice and Rats Produced by Neoprontosil, Sulfanilamide, Sodium Sulfapyridine and Sodium Sulfathiazole.

A. James French. (Introduced by C. V. Weller.)

From the Department of Pathology, University of Michigan, Ann Arbor, Mich.

In the routine examination of autopsy material at the University Hospital, examples of interstitial myocarditis, in which the cellular infiltrations included eosinophile cells in varying numbers, have been encountered with increasing frequency in recent years. After excluding all cases with trichinosis, rheumatic heart disease, demonstrable septicopyemia and other recognized causes of interstitial myocarditis, there was left a group in which the only factor possessed in common was that each patient had received medication with a drug of the sulfonamide group.

Dozzi,[1] and Scheinberg and Ingle[2] have independently described electrocardiographic changes suggestive of myocardial damage following the use of sulfonamide drugs. No other reference to myocarditis resulting from such medication has been found in a careful search of the literature, save for the observation by Nelson[3] of myocarditis, with infiltrating polymorphonuclear and monocytic cells, in a hen to which sulfanilamide had been administered.

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[1] Dozzi, L., *Am. J. Med. Sci.*, 1938, **195**, 771.

[2] Scheinberg, D., and Ingle, C. W., *Memphis Med. J.*, 1939, **14**, 87.

[3] Nelson, A. A., *Pub. Health Rep.*, 1939, **54**, 106.

As a preliminary procedure, groups of rats (Wistar strain), Swiss mice and domestic rabbits were given neoprontosil by the daily intraperitoneal injection of an amount computed to be comparable to the usual clinical dosage (4 g per 24 hours for a man weighing 75 k). The following volumes of a 5.0% solution of neoprontosil were used: Mouse, 0.015 cc; rat, 0.15 cc; rabbit, 1.5 cc. Interstitial cellular infiltrations were found in many of these animals. Accordingly larger groups of animals were employed and sulfanilamide, sodium sulfapyridine and sodium sulfathiazole were employed as well as neoprontosil. The daily dosage is shown in Table I.

The mice and rats were killed at intervals of 10, 20 and 30 days. The rabbits were treated for but 10 days since sodium sulfathiazole and sodium sulfapyridine were not available in sufficient quantities for protracted use, and it seemed desirable to use the sodium salts because of their greater solubility. To smaller groups of mice and rats doses 10 times the computed clinical amount were given. The combined results follow: 68 mice treated, 38 showed myocarditis; 56 rats treated, 42 showed myocarditis; 20 rabbits treated, 17 showed myocarditis. Myocardial infiltrations followed the use of each of the 4 drugs administered. No significant differences among the 4 as to the danger of producing myocarditis were revealed. In general, the frequency of myocarditis increased with the duration of treatment and with increased dosage.

As controls, 29 mice, 26 rats and 26 rabbits from the same stocks of animals, all bred, housed and fed in the same manner as those treated, were killed and their hearts examined microscopically. "Spontaneous" myocarditis[4] was found in several of the control rabbits. The results in the treated rabbits were therefore excluded from further consideration. No cellular infiltrations were found in the hearts of either the control rats or mice.

Summary. In the mouse and rat an interstitial myocarditis, of which the cellular infiltration is in part eosinophilic, results from the

TABLE I.

	% Sol.	Mouse, cc	Rat, cc	Rabbit, cc
Neoprontosil	5.0	.015	.15	1.5
Sulfanilamide	0.5	.15	1.5	15.0
Sodium sulfapyridine	5.0	.015	.15	1.5
Sodium sulfathiazole	5.0	.015	.15	1.5

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[4] Miller, C. P., *J. Exp. Med.*, 1924, **40**, 543.

intraperitoneal injection of neoprontosil and certain other drugs of the sulfonamide group. This myocarditis is histologically similar to that observed in the hearts of humans dying after the administration of these drugs.

13201

Biotin and Scaly Dermatosis of the Chick.

S. Ansbacher and Maurice Landy.

From the Division of Experimental Medicine, The Squibb Institute for Medical Research, New Brunswick, N.J., and the Research Laboratories, S. M. A. Corporation, Chagrin Falls, Ohio.

In a recent communication,[1] chicks raised on a heated diet[2] were reported to show symptoms of a severe dermatosis similar to the one described by Hegsted, *et al.*[3] As shown in Tables I and II, the deficiency syndrome was readily obtained with chicks reared on diet X-5, which is ration K-11[4] modified by the addition of 2 mg of 2-methyl-1,4-naphthoquinone per 10 kg of diet. The symptoms most frequently developed on the toes and feet, often in the cor-

TABLE I.
Effect of Biotin Concentrates on Growth and Scaly Dermatosis in Chicks.*

Depletion period Wt and symptoms†						Test period Wt and symptoms†		
26th day, g	55th day, g	68th day, g	Daily doses, γ	Route‡	No. of chicks	8th day, g	17th day, g	25th day, g
130 N	230 D	290 M	0	O	20	340 S	——	350 S
160 N	270 M	350 S	2.5	O	5	440 DH	530 C	550 C
130 N	220 M	260 S	1.25	O	5	330 DH	410 C	440 C
140 N	210 M	230 S	24	SC	4	290 DH	340 C	350 C
150 N	250 M	290 S	18	SC	4	360 DH	440 C	480 C
140 N	250 M	280 S	12	SC	4	360 DH	460 C	500 C
130 N	270 M	310 S	6	SC	4	410 DH	530 C	600 C
130 N	210 M	250 S	2.5	SC	3	340 DH	440 C	490 C

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[1] Ansbacher, S., *Science,* 1941, **93**, 164.

[2] Ansbacher, S., Proc. Soc. Exp. Biol. and Med., 1940, **44**, 248.

[3] Hegsted, D. M., Oleson, J. J., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Nutrition,* 1940, **20**, 599.

[4] Ansbacher, S., Proc. Soc. Exp. Biol. and Med., 1941, **46**, 421.

TABLE II.
Effect of Crystalline Biotin on Growth and Scaly Dermatosis in Chicks.*

Depletion period Wt and symptoms†			Doses			Test period Wt and symptoms†	
25th day, g	42nd day, g	64th day, g	γ	No. of	Route‡	8th day, g	16th day, g
110 N	200 D	270 S	10	3	O	330 DH	440 C
140 N	200 M	280 S	10	3	O	280 DH	460 C
150 N	250 D	390 S	3	5	IM	390 DH	450 C
120 N	210 D	320 M	5	3	IM	400 DH	590 C
100 N	140 M	220 S	10	3	IM	220 DH	360 C
100 N	200 D	230 S	10	3	IM	290 DH	410 C
120 N	210 D	300 M	10	3	IM	360 DH	460 C

*Chicks reared on diet X-5:	%
Heated [120°C, 1 week] grain [25 parts wheat middlings and 58 parts yellow corn] mixture	83
Salt mixture [Table I, *J. Nutrition*, 1939, **17**, 303]	2
Calcium carbonate	1
Cod liver oil containing 10 ml menadione	
Casein [SMACO, vitamin-free] with added 80 mg thiamine hydrochloride, 80 mg riboflavin, 80 mg pyridoxine hydrochloride, 1 g calcium pantothenate, 1 g nicotinic acid, 1 g inositol, 3 g choline chloride and 3 g p-aminobenzoic acid per 1.2 kg	12

†Symptoms: N—None; D—Definite; M—Marked; S—Severe; DH—Definite Healing; C—Complete Healing.

‡Route: O—Oral; SC—Subcutaneous; IM—Intramuscular.

ners of the beaks and on the combs, occasionally on the eyelids, and were always most severe on the toes.

Biotin concentrates (SMACO) were found to possess growth-promoting property and curative effect (Table I), all the treated animals showing definite improvement within a period of 5 days and practically complete healing of the scaly dermatosis in about 2 weeks. Similar results (Table II) were obtained with crystalline biotin methyl ester,[5] kindly supplied by Dr. Vincent du Vigneaud. It should be mentioned that the treated birds showed at the end of the test period a residual dermatosis on the toes, the extent of which was in many cases so slight that a careful examination was necessary to find it.

The data presented show conclusively that the scaly dermatosis observed in the chicks is a result of a simple[6] dietary deficiency in biotin. Concentrates fed daily at a level equivalent to only 1.25γ of biotin were effective. Severely depleted birds responded to crude preparations subcutaneously injected in daily doses equivalent to as little as 2.5γ of biotin. The scaly dermatosis was observed to be healed by the administration of the crystalline biotin ester. The minimum curative dose has not yet been determined and is probably less than 10γ.

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[5] György, P., Rose, C. S., Hofmann, K., Melville, D. B., and du Vigneaud, V., *Science*, 1940, **92**, 609.

[6] György, P., *Arch. Dermat. and Syphil.*, 1941, **43**, 230.

It is apparent from the experiments as a whole that the chick can be employed in a quantitative biological assay of biotin. Such a procedure is certainly more time-consuming than, *e. g.*, a yeast growth technic,[7] but it is definitely more expedient than a bioassay with rats based upon a secondary[8] deficiency disease brought about by egg-white injury.

Conclusion. Biotin-deficiency is brought about in the chick reared on a heated ration of low biotin content ($0.1\gamma/g$). This deficiency is produced in the absence of dietary egg white which is essential for the development of the avitaminosis in the rat. Deficiency symptoms are completely cured by administration of crystalline biotin methyl ester. The chick can be used in a quantitative biological assay of biotin.

13202

Generalized Muscular Weakness as a Toxic Reaction to Digitalis.*

John S. La Due. (Introduced by A. D. Hirschfelder.)

From the Department of Medicine, The University of Minnesota Medical School, Minneapolis.

Clark,[1] Cushny[2] and Neuschloss[3] demonstrated that the efficiency of a nerve muscle preparation could be markedly impaired if excessive amounts of a cardiac glycoside were added to the perfusing fluid. The work capacity of the skeletal muscle preparations was temporarily increased by small amounts of digitalis, reduced by excessive amounts, and returned to normal after the drug was removed.

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[7] Snell, E. E., Eakin, R. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1940, **62**, 175.

[8] György, P., Rose, C. S., Eakin, R. E., Snell, E. E., and Williams, R. J., *Science*, 1941, **93**, 477.

* This paper comprises part of a thesis submitted to the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Medicine.

[1] Clark, A. J., *Brit. Med. J.*, 1912, **2**, 687.

[2] Cushny, A. R., The Action and Uses in Medicine of Digitalis and Its Allies, Longmans, Green and Co., London, 1925.

[3] Neuschloss, S. M., *Pflugers Arch. f. d. ges. Phys.*, 1922, **197**, 235.

The toxic reactions of patients to cardiac glycosides have been studied and documented since the publication of Withering's[4] monograph in 1785, but careful search of the literature has failed to reveal any described clinical instance of digitalis affecting skeletal muscle. The writer believes that the following case history illustrates toxic changes in skeletal muscle resulting from the administration of large amounts of digitalis.

Case History. A 23-year-old girl who had aortic insufficiency and mitral stenosis of rheumatic origin developed dyspnea and transient edema during September, 1935. In June, 1936, she was hospitalized and was given a total amount of 22 grains of USP X digitalis (1½ grain = 1 Hatcher-Brody cat unit) in 36 hours, followed by a maintenance dose of 3 to 4 1½ grain tablets. After leaving the hospital she continued to take the drug daily in this dosage until September, 1940. During this period, the digitalis was changed from USP X to USP XI potency, an increase of from 30 to 60%. This represents a maintenance dose 3 to 5 times greater than that usually given.

Except for moderate dyspnea on exertion, she was symptom free until June, 1939, when she first noticed severe cramping in the muscles of both legs. Soon after this she found that slight exertion was accompanied by marked fatigue and weakness and she was unable to climb a single flight of stairs without great effort. Weakness was most noticeable in the leg and back muscles, but she stated that her arms "felt heavy" and that she could not hold a book in one position for long. These symptoms were so marked that she could not return to college in the fall and was confined to the house most of the time. Cramping of the leg and back muscles occurred almost every day. In September, 1939, she experienced occasional episodes of double vision coming on in the evening or after prolonged use of her eyes.

Except for occasional brief remissions, these symptoms persisted until September, 1940, and were the only symptoms noticed by the patient. There were no anorexia, nausea, emesis or palpitation during this 4-year period when she was taking 3 to 4 cat units of digitalis daily.

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[4] Withering, W., An Account of the Foxglove and Some of Its Medical Uses: with Practical Remarks on Dropsy and Other Diseases, M. Swinney, Birmingham, 1785.

When the writer first examined the patient in September, 1940, the heart was generally enlarged, with an ml of 13.4 cm and an mr of 4.7 cm. The rhythm was regular at 42; the first sound was loud and snapping, followed by a rough, whistling systolic murmur. P_2 was split, accentuated and followed by a soft, high-pitched diastolic murmur best heard along the left sternal margin. The blood pressure was 130/70; the lungs were clear and resonant; the liver was not palpable and there was no peripheral edema.

The neurological examination was essentially within normal limits except for a demonstrable weakness of all the skeletal muscles. The grip was weak, the patient was unable to raise herself from a prone to a sitting position without the use of her hands and, despite firm, well-developed musculature, weakness was apparent in all muscle actions. She could not jump up and down on one foot or lift her weight by her arm and shoulder muscles. She was asked to climb one flight of 36 four-inch steps as rapidly as possible and this took 7½ minutes with frequent rest periods. The exertion was not accompanied by dyspnea, but the pulse rose to 56 and was irregular. The electrocardiogram showed complete heart block, auricular fibrillation and right axis deviation.

Digitalis was discontinued and the patient was examined twice daily. For 2 days she still noted muscle pain and weakness, but thought them somewhat less severe. On the third day she felt much stronger and had no pain. Four days after the discontinuance of digitalis, she climbed the same flight of stairs in one minute, and was able to jump up and down on one foot 10 times, could rise from her back to a sitting position without the help of her hands and had a much stronger grip. At the end of a week, she stated that she felt stronger than ever before in her life.

Heart block persisted for one week and was followed by an absolutely irregular ventricular rate (55-65) which lasted 2 weeks. Then the rate rose above 70 and lanatoside C (a crystalline cardiac glycoside) was given in dosage of one Hatcher-Brody cat unit (0.25 mg) daily. The patient returned to college in January, 1941, and has had no further symptoms.

The writer had previously noted[5] that experimental administration of daily intravenous doses of lanatoside C to dogs (1/5 to ½ cat unit per kilo) resulted in marked weakness after 2 to 3 weeks. This was interpreted as being due to starvation, since the animals had severe anorexia and vomited frequently. However, the case history reported here suggested a different interpretation of the muscular weakness of these animals and prompted further investigation.

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[5] LaDue, J. S., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 651.

Four dogs were trained to run on a treadmill until they were able to exercise for more than 30 minutes without any sign of fatigue. Then daily intravenous injections of 1/5 of a cat unit of lanatoside C per kilo of body weight were given.[†] After 2 weeks these animals exhibited increasing anorexia, occasional emesis, sialorrhea and progressive muscular weakness. On the 23rd day, one animal collapsed as soon as the treadmill was started, 2 could not run more than 5 minutes without falling on the 24th day of the experiment, and the remaining dog was unable to exercise on the 25th day. The drug was then discontinued and food was withheld. Within 4 days, the dogs were again able to exercise 20 to 30 minutes. Feedings were resumed and within 2 days the animals were able to exercise as well as at the beginning of the experiment.

Summary and Conclusions. 1. A case history illustrating generalized muscular weakness as a toxic effect of digitalis is presented. 2. Four dogs given daily injections of a cardiac glycoside developed generalized muscular weakness. Four days after discontinuance of the drug, the animals were able to exercise without fatigue. 3. With the present trend towards increased digitalis dosage, it is possible that this syndrome may be encountered by other investigators.

[†] This dosage was equivalent to about twice the maintenance dose which the patient had been taking.

13203

Is Galacturonic Acid Absorbed by the Small and Large Intestine?

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Galacturonic acid is one of the decomposition products of pectin. Manville, Bradway and McMinis have reported results in the rabbit which indicated that, on feeding pectin, galacturonic acid is produced in and absorbed from the intestine and eliminated as a galacturonide in the urine.[1] Malyoth[2] first made such a suggestion, but later[3] stated, without supplying data and methods, that a high percentage of galacturonic acid, when given by mouth or parenterally to experimental animals, is recovered in the excreta without being conjugated. Our work was done to ascertain to what extent galacturonic acid is absorbed by the human and canine intestine and canine colon.

Methods. The galacturonic acid used was obtained from the Research Department of the California Fruit Growers Exchange. By the Link Method[4] the preparation yielded 21.8% CO_2, which is equivalent to 87.2% uronic anhydride. By the A. O. A. C. method[5] for the determination of furfural, one gram of the preparation yielded 0.300 g of phloroglucide.

Under nembutal anesthesia, a loop of ileum and colon was prepared by the usual method in 6 dogs. In addition, 3 unanesthetized dogs with permanent Thiery-Vella fistula of the ileum and one with a permanent entirely isolated colon fistula were used. Two healthy human subjects with an ileostomy and a snugly fitting ileostomy bag volunteered for the study. In all tests a 5% solution of galacturonic acid having a Δ of $-0.56°C$ and a pH of 2.5 was used; it was isotonic with erythrocytes. In the tests on the anesthetized dogs and on the dog with colon fistula, the absorption period was 2 hours, and 50 cc of the solution was introduced. In the case of the Thiery-Vella loops an absorption period of 1 hour was used, and 25, 40 and 50 cc of the solution was introduced. In all cases the loop was irrigated with Ringer's solution before the galacturonic acid was introduced. At the end of the absorption period the loop was drained and irrigated with two 20 cc volumes of Ringer's solution. The washings were added to the fluid drained from the loop after its volume was measured, and the total volume analyzed in duplicate portions. The human subjects reported to the laboratory without breakfast and without taking water since the previous evening. As a control, they were given 200 cc of distilled water, and the discharge that passed into the ileostomy bag was collected at the end of 2 hours and analyzed for galacturonic acid, in duplicate portions. Then 200 cc of the galacturonic acid solution was ingested and the discharge collected and assayed at the end of 2 hours, when the flow from the fistula had practically ceased. Any uronic acid found in the control discharge was subtracted from the values obtained from the discharge obtained after the ingestion of the galacturonic acid.

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[1] Manville, I. A., Bradway, E. M., and McMinis, A. S., *Am. J. Dig. Dis. and Nutr.*, 1936, **3**, 570.

[2] Malyoth, G., *Klin. Wchnschr.*, 1931, **10**, 1159, 1255.

[3] Malyoth, G., *Klin. Wchnschr.*, 1934, **13**, 51.

[4] Dickson, A. D., Otterson, H., and Link, K. P., *J. Am. Chem. Soc.*, 1930, **50**, 775.

[5] *Method of Analysis,* Association of Official Agricultural Chemists, p. 344, 4th Ed., 1935.

All the assays included analysis by the 2 chemical methods indicated above.

Results. Recovery of galacturonic acid from the ileum and colon, in the 6 anesthetized animals, after a 2-hour absorption period, was practically the same. For the ileum it was 93% both by the phloroglucide and uronic acid methods, while for the colon it was 91% by the phloroglucide and 94% by the uronic acid method. In these experiments the volume of clear fluid drained at the end of the test period was practically the same as that which was introduced. Recovery from the 3 unanesthetized, Thiery-Vella dogs (9 experiments) was also very much the same for both the ileum and colon. In the case of the ileum it was 90% by the phloroglucide method and 94% by the uronic acid method. The volume of clear fluid drained from the ileum was greater than that introduced, in every instance, while in the 6 colon experiments, the volume of the fluid drained was smaller than that introduced.

From the human ileostomy subjects the average recovery of galacturonic acid was 91% by the phloroglucide method and 95% by the uronic acid method. The volume of fluid in the ileostomy bags was less than that taken in all of the control experiments, and more in all of the test experiments. The control volumes averaged 83 cc and the test volumes averaged 427 cc. The pH of the recovered fluid in all the foregoing tests was found to be approximately 7 when tested by the glass electrode.

The average recovery of the introduced galacturonic acid ranged from 87 to 93% by the phloroglucide method, and 92 to 95% by the uronic acid method of Link. Due to the inherent errors in the methods, and the possibility that some of the galacturonic acid may have been destroyed by bacterial action, it is doubtful whether any of the galacturonic acid was absorbed.

Conclusions. Less than 10%, if any, of galacturonic acid, introduced as an isosmotic and isotonic solution, is absorbed from the canine small and large intestine and the human small intestine. Isosmotic galacturonic acid stimulates the secretion of fluid by the small intestine of unanesthetized human and canine subjects.

13204

Effect of Androgen Administration upon Pregnandiol Excretion in Cyclical Women.

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From the Laboratories, Mount Sinai Hospital, New York City.

There is abundant evidence in the studies of Venning and Browne[1, 2] and others,[3, 4, 5] which indicates that the progesterone which is formed in the corpus luteum of cyclical women is excreted in the form of sodium pregnandiol glucuronidate (SPG). In previous communications, we have reported that testosterone propionate, administered in sufficient amounts to cyclical women, suppresses follicle growth, ovulation and corpus luteum formation,[6] inhibits production of estrogen and progesterone by the ovary[7, 8] and, as a consequence, prevents the development of the secretory pattern of the endometrium, ultimately leading to atrophy of the endometrium[7, 8, 9] and vaginal mucosa.[10, 11]

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[1] Venning, E. H., and Browne, J. S. L., *Am. J. Physiol.*, 1937, **119**, 417.

[2] Venning, E. H., and Browne, J. S. L., *Endocrinology*, 1937, **21**, 711.

[3] Hamblen, E. C., Ashley, C., and Baptist, M., *Endocrinology*, 1939, **24**, 1.

[4] Stover, R. F., and Pratt, J. P., *Endocrinology*, 1939, **24**, 29.

[5] Wilson, R. B., Randall, L. M., and Osterberg, A. E., *Am. J. Obs. and Gyn.*, 1939, **37**, 59.

[6] Geist, S. H., Gaines, J. A., and Salmon, U. J., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 319.

[7] Geist, S. H., Salmon, U. J., Gaines, J. A., and Walter, R. I., *J. A. M. A.*, 1940, **114**, 1539.

[8] Salmon, U. J., *J. of Clin. Endocrinology*, 1941, **1**, 162.

[9] Gaines, J. A., Salmon, U. J., and Geist, S. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 779.

[10] Salmon, U. J., Walter, R. I., and Geist, S. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 467.

[11] Salmon, U. J., Geist, S. H., and Walter, R. I., *Am. J. Obs. and Gyn.*, 1939, **38**, 264.

The evidence pointing to inhibition of corpus luteum development and suppression of progesterone formation consisted of (a) the failure to find a corpus luteum in the ovaries of cyclical women treated with testosterone, at a time when one would, under normal conditions, expect to find one;[6] and (b) the failure to find a secretory pattern in the premenstrual endometrium of these women.[7, 9] As part of a series of studies of the biologic properties of androgens in the human female, we investigated the effect of androgen administration upon excretion of the pregnandiol complex.

Methods and Material. A group of 8 women with regular menstrual cycles, in whom premenstrual endometrial biopsies revealed typical secretory patterns indicative of normal estrogen-progesterone production, were given androgens. SPG determinations were performed according to the method of Venning,[12, 13] during periods of the cycle indicated in Table I. At the termination of the experimental study, 3 of these patients were operated upon and the ovaries examined grossly and microscopically for corpora lutea.

The androgens used were: (1) methyl testosterone (3 patients), given orally, in daily doses of 50 to 150 mg (total doses of 1480, 2350 and 2800 mg); (2) testosterone propionate in oil, administered intramuscularly (3 patients), in individual doses varying from 50 to 100 mg (total doses of 600, 1050, and 1175 mg); (3) testosterone (500 mg) and testosterone propionate (516.6 mg) pellet implantation (2 patients).

TABLE I.

					Pregnandiol excretion		
Case No.	Androgen	Dose, mg	Route of administration	Time of administration, days of cycle	Amount, mg	Time of studies, days of cycle	Remarks
1	m.t.	1480	oral	1-21	0	1-40	M.P. suppressed.
2	m.t.	2350	”	1-24	0	4-24	M.P. suppressed. No c.l. found at operation.
3	m.t.	2800	”	1-30	0	6-30	M.P. suppressed.
4	t.p.	600	intramuscular	1-14	0	6-30	M.P. suppressed. No. c.l. found at operation.
5	t.p.	1050	”	1-23	0	6-34	M.P. suppressed. No c.l.
6	t.p.	1175	”	10-21	55	14-22	M.P. occurred on 24th day of cycle.
7	t.p.	516.6	pellet implantation	1	0	1-43	M.P. suppressed.
8	t.	500	” ”	4	0	4-30	M.P. suppressed.

m.t.—methyl testosterone. t.p.—testosterone propionate. t.—testosterone. M.P.—menstrual period. c.l.—corpus luteum.

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[12] Venning, E. H., *J. Biol. Chem.*, 1937, **119**, 473.

[13] Venning, E. H., *J. Biol. Chem.*, 1938, **126**, 595.

Results. Methyl Testosterone. No SPG was found in any of the patients during the current cycle when the androgen was being administered. In each case, the expected menstrual period was suppressed. The pregnandiol determinations were performed beyond the time when the pregnandiol complex would normally be found. In one case, the studies were conducted for 40 consecutive days. One patient of this series (Case No. 2) was operated on at the end of the experiment. The ovaries did not contain a current corpus luteum.

Testosterone Propionate. In this group, one patient was given testosterone propionate during the first 2 weeks (Case No. 4), one during the first 3 weeks (Case No. 5), and a third during the middle 10 days of the cycle (Case No. 6). In the first two, no SPG was found in the urine, whereas a total of 55 mg was recovered from the urine of the third patient. Cases No. 4 and No. 5 were both operated on and no corpus luteum was found in either.

It is noteworthy that in Case No. 6. in which 55 mg of SPG was found, the testosterone propionate was administered after the 9th day of the cycle when, presumably, the hypophyseal gonadotropic factor had already been secreted. Apparently, the administration of even so large a dose as 1175 mg of testosterone propionate could not suppress progesterone production if given during the middle of the cycle. If the testosterone is administered during the first 2 weeks of the cycle, a much smaller dose (*e. g.,* 600 mg) will prevent pregnandiol excretion (Case No. 4). This is in agreement with previous observations that 500 to 600 mg of testosterone propionate is sufficient to suppress ovulation and menstruation if administered during the first 2 weeks of the cycle, but has no such inhibitory effect on the current cycle if the same or larger doses are administered during the last 2 weeks of the cycle.[7, 8]

Androgen Implantation. In the 2 patients implanted, one with 516.6 mg of testosterone propionate on the first day of the cycle (Case No. 7) and the other with 500 mg of testosterone on the 4th day of the cycle (Case No. 8), the SPG was completely absent from the urine for 42 and 26 days, respectively, after the implantation. The testosterone propionate pellet (Case No. 7) was excised 63 days after the implantation and was found to have lost 306.6 mg representing an average daily absorption of 4.9 mg. At this rate, the patient absorbed approximately 137.0 mg during one cycle. The testosterone pellet was excised 58 days after the implantation and was found to have lost 344 mg, representing an average daily absorption of 5.9 mg. During the current cycle (24 days) this would represent an absorption of 141.6 mg of testosterone. Previous studies have shown that, when administered intramuscularly in solution in sesame oil, the absorption is very rapid and consequently a considerable amount of the hormone is excreted before it can produce any effect.

Summary and Conclusions. Eight women with regular menstrual periods and normal cyclic estrogen and progesterone production, as evidenced by the finding of typical secretory endometria premenstrually, were treated with androgens (methyl testosterone by mouth, testosterone propionate by injection, and testosterone and testosterone propionate by implantation) in order to determine the effect of the androgens upon excretion of SPG. The results indicate that doses of androgens which have been found, in previous studies, to be suppressive (*i. e.*, to cause suppression of pituitary gonadotropic hormone excretion; inhibition of ovulation and menstruation), when administered early in the cycle, result in failure of SPG to appear in the urine during the current cycle and lead to suppression of the next expected menstrual period. In one patient (Case No. 6), 1175 mg of testosterone propionate, administered after the 9th day of the cycle, did not appear to appreciably affect the excretion of the pregnandiol complex during the current cycle.

These results are interpreted as indicating that if sufficient androgens are administered early in the cycle, before the pituitary gonadotropin has been secreted or had time to exert its effect on the ovary, progesterone production is suppressed, resulting in absence of pregnandiol from the urine during the current cycle. That absence of SPG from the urine is due to failure of progesterone formation and not to interference with the conversion of progesterone to the pregnandiol complex is proven by the absence of corpora lutea from the ovaries in the patients with no pregnandiol excretion.

13205

Some Aspects of Metabolism Following Parenteral Administration of Casein Hydrolysate.

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During the process of digestion of proteins, amino acids are liberated and pass unchanged into the portal circulation. A portion of these amino acids is utilized as a source of energy while another is used by the body for the formation of many characteristic tissue proteins.[1] According to Rose the indispensable amino acids are as follows: lysine, tryptophane, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine and arginine. (The indispensability of arginine is questioned.) Of these amino acids l-tryptophane, l-histidine, l-phenylalanine, and l-methionine can be replaced for growth purposes by their optical isomers.

This paper deals with: (a) A method for the preparation of a hydrolysate of casein suitable for parenteral administration to humans; and (b) its effect on urea-N and amino acid-N of the blood of fasting rabbits.

Methods. Blood amino acid-nitrogen was determined by Sahyun's methods,[2] and blood urea nitrogen by Wrenn's.[3]

Experimental. The effects of sulfuric acid and of barium hydroxide were studied on each of the amino acids listed in Table I. A 100-mg sample of each amino acid was treated with 5 N sulfuric acid and 40% commercial barium hydroxide respectively. The samples were autoclaved at 20 lb steam pressure for 12 hours. Each sample was then made alkaline to phenolphthalein, filtered, and aliquot samples of the filtrate immersed in a boiling water bath to drive off any ammonia that might have been formed. Nitrogen was then determined. The results are given in Table I.

Of the amino acids studied cystine and arginine suffered either loss or destruction by treatment with barium hydroxide. A portion of lysine was destroyed, while tryptophane was completely destroyed by treatment with sulfuric acid.

Procedure A—Acid Hydrolysis. Into a 20-liter Pyrex bottle

TABLE I.
Effect of Autoclaving for 12 hr at 20 lb Steam Pressure of Certain Amino Acids in the Presence of 5 N Sulfuric Acid and 40% Barium Hydroxide.
Mg Nitrogen per Sample.

		Autoclaving with	
Amino acid	Amount, mg	Barium hydroxide, mg	Sulfuric acid, mg
Lysine	6.8	6.5	4.8
Tryptophane	13.7	11.2	5.6
Arginine	26.0	11.0	No change

Histidine, phenylalanine, leucine and valine were not affected by autoclaving with either barium hydroxide or sulfuric acid.

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[1] Rose, W. C., *Physiol. Rev.*, 1938, **18**, 109.

[2] Sahyun, M., *J. Lab. Clin. Med.*, 1939, **24**, 548.

[3] Wrenn, H., *J. Lab. Clin. Med.*, 1937, **22**, 1040.

10 liters of 5 N sulfuric acid were introduced, then 2 kg of casein were added slowly while stirring. The bottle was then covered with a piece of cheesecloth and placed in an autoclave. Steam pressure was gradually increased until it reached 20 lb per square inch. Autoclaving was continued for about 16 hours. The content of the bottle was allowed to cool over night, then calcium oxide (slacked lime) was added until the reaction was decidedly alkaline to phenolphthalein. Distilled water was then added until the volume was about 30 liters. The hydrolysate was allowed to stand over night until the calcium sulfate had settled, then the clear supernatant liquid was siphoned off. The calcium sulfate precipitate was filtered, washed with hot distilled water, and the washings were added to the filtrate. To remove ammonia the alkaline filtrate was next concentrated in vacuum to about 10 liters. The ammonia-free filtrate was next saturated with carbon dioxide and a considerable portion of the excess calcium was removed as calcium carbonate. This was filtered off and oxalic acid was added to the clear filtrate to precipitate out most of the remaining calcium. The liquid was heated, filtered, and to the clear filtrate barium hydroxide was added to precipitate the last traces of sulfate present in the hydrolysate. The addition of barium hydroxide was carefully controlled so that a filtered sample gave but a slight test for sulfate, thus the absence of barium was assured. As a rule, the amount of sulfate did not exceed 5 to 10 mg calculated as sulfuric acid per 100 cc. Nitrogen was next determined. The final preparation was either concentrated in vacuum or diluted with pyrogen-free doubly-distilled water to obtain a desired concentration. The acidity was adjusted to about pH 5. l-Tryptophane and cystine were added to the extent of 1.0% of the total amino acids present calculated on the basis that a solution of the mixed amino acids containing 2.1% nitrogen would yield about 15% amino acids. (From this concentrate suitable dilutions of 5% and 10% were made for clinical and animal experimentation.) This mixture was filtered through a clarifying pad, sterilized by passing it through a sterile Berkefeld filter, and stored in sterile bottles in the cold at about 4°C.

Procedure B—Acid-Base Hydrolysis. One kg of casein was hydrolyzed in 5 N sulfuric acid by autoclaving for about 16 hours as described above; simultaneously another one kg was hydrolyzed in 5 liters of 40% barium hydroxide. Hydrolysis was judged complete when samples yielded a negative biuret test. Then, while stirring, the sulfuric acid hydrolysate was slowly added to the barium hydrolysate. The reaction of the mixture was next made alkaline with barium hydroxide using phenolphthalein as an indicator. Ammonia was removed by concentration of the mixture in vacuum to about half of the original volume. The excess of barium was removed by the addition of sulfuric acid so that a filtered sample gave but a faint sulfate test. This material was filtered and about 6 to 8 g of oxalic acid was added to the clear filtrate to precipitate any excess calcium found in the solution from the hydrolyzed casein. The preparation was adjusted so that the final nitrogen content was 2.1%, which would yield about 15% amino acids. (From this concentrate suitable dilutions were made for clinical and animal experimentation.) This mixture of amino acids was termed "acid-base hydrolysate" of casein. It is to be remembered, however, that about one-half of the amino acids in the final preparation consisted of racemized amino acids.

Experiment on Rabbits. The following solutions were made in doubly distilled water: (1) A 10% solution of glycine, (2) a 10% solution of acid hydrolysate "A" with added tryptophane and cystine, and (3) a 10% solution of acid-base hydrolysate "B". Ten cc per kg body weight of each of the 3 preparations were subcutaneously administered into each of a group of 10 rabbits that had fasted for 24 hours. Samples of blood were withdrawn at 0, 1.5, 3, 5, and 7 hour intervals respectively. Urea-N and amino acid-N were determined. The averages of the results for this experiment are reported in Figs. 1 and 2.

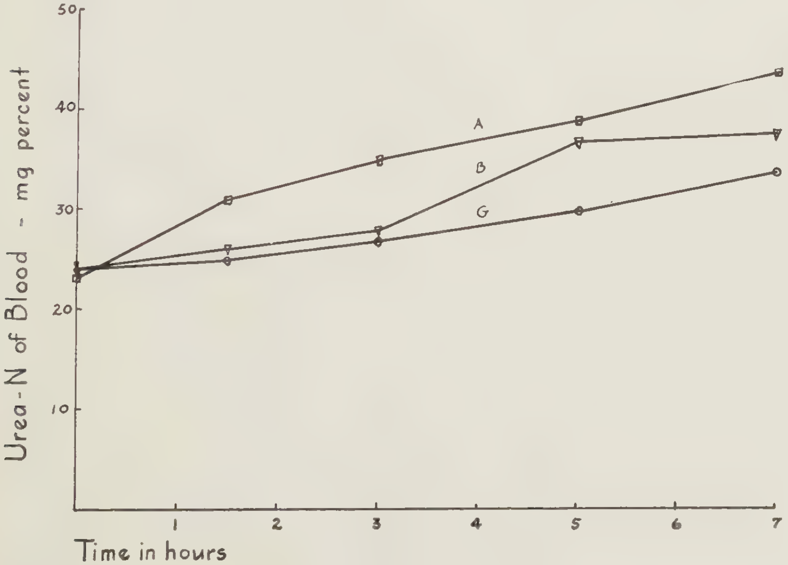

FIG. 1.
Blood Urea Nitrogen.

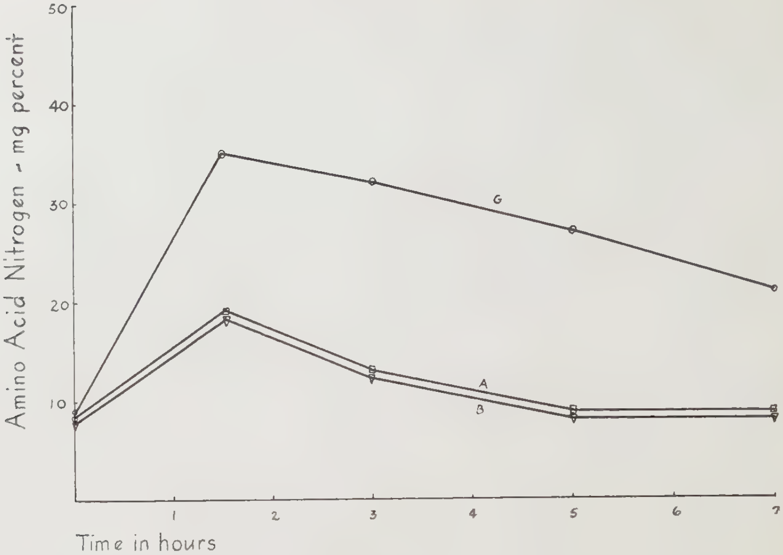

FIG. 2.
Blood Amino Acid Nitrogen.

Following the effects of the subcutaneous administration into fasting rabbits of 10 cc of a 10% solution per kg body weight of : (A) Acid hydrolysate of casein with added tryptophane and cystine, (B) Acid-base hydrolysate, and (G) Glycine. Each curve represents the averages of the results obtained on 10 rabbits.

Discussion. The data presented on the effect of glycine on amino acid nitrogen, and urea nitrogen of blood are in harmony with the earlier work of Lewis and Izume,[4, 5] and of Blatherwick, Sahyun, and Hill.[6] The experiment on the administration of glycine was performed to serve as a basis of comparison between the administration of a single amino acid and a mixture of amino acids. The results obtained indicated very clearly that the administration of a mixture of amino acids caused a more rapid deaminization as was shown by the faster rate of urea formation and smaller accumulation of amino acid nitrogen in the blood as compared with the figures obtained following the administration of glycine. The considerable increase in blood amino acid nitrogen observed (Fig. 2) following the injection of glycine may be attributed in part or *in toto* to the fact that the dose of glycine was about twice as great in respect to NH_2-N as doses of amino acid mixtures because of the higher average of molecular weight of the latter.

Following the administration of the acid-base hydrolysate the results obtained showed a less marked rate of deaminization as com-

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[4] Lewis, H. B., and Izume, S., *J. Biol. Chem.*, 1926, **71**, 33.

[5] Izume, S., and Lewis, H. B., *J. Biol. Chem.*, 1926, **71**, 51.

[6] Blatherwick, N. R., Sahyun, M., and Hill, E., *J. Biol. Chem.*, 1927, **75**, 671.

pared with the result obtained following the administration of the acid hydrolysate to which tryptophane and cystine had been added. This difference in action between the two types of casein hydrolysate used in this experiment may be explained on the basis that in the acid-base hydrolysate almost half of the amino acids administered were in the racemic form due to the procedure employed in hydrolyzing 50% of the casein with barium hydroxide.

Summary. A method for the preparation of a casein hydrolysate suitable for parenteral administration is described. Data are presented showing greater utilization and deaminization following the parenteral administration into fasting rabbits of an acid hydrolysate of casein to which l-tryptophane and l-cystine had been added, than following the administration of either glycine or partially racemized amino acids of an acid-base hydrolysate of casein.

The author is indebted to Arthur Nixon and Andrew Pangrace for valuable assistance.

13206

Enhancing Effect of Nicotinic Acid and Cysteine Hydrochloride on Growth of *Leptospira icterohaemorrhagiae*.*

THOMAS G. WARD AND ELIZABETH B. STARBUCK. (Introduced by Thomas B. Turner.)

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While carrying out various studies on *L. icterohaemorrhagiae,* the difficulty of growing these organisms in quantities suitable for use as antigen in the agglutination or complement fixation tests constantly presented itself. Likewise, some difficulty had been encountered in perpetuating strains of leptospira in culture media, since they died for no apparent reason, and batches of media prepared identical to previous ones would often not sustain the growth of these organisms.

In an attempt partially to resolve these difficulties, the effect of the addition of various amino acids and nicotinic acid to a modified Noguchi semi-solid medium was tested.

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* These studies were supported in part by a grant from the International Health Division of the Rockefeller Foundation.

Experimental Methods. The basic medium used in these studies was prepared as follows: To a mixture of 10 cc normal rabbit serum, 1.5 cc hemoglobin (1 part rabbits' blood to 3 parts sterile distilled water) and 68.5 cc normal saline was added 10 cc of a previously melted 2% infusion agar. To this basic medium was later added 10 parts of the solution containing the growth factor to be tested.

The solutions to be added were prepared by dissolving 50 mg of nicotinic acid or one of the amino acids in 50 cc of normal saline. The solution was sterilized by filtration through a Seitz pad. Eight solutions, containing, respectively, nicotinic acid, cysteine hydrochloride, tyrosine, valine, glutamine, leucine, glycine, and methionine were prepared, and 10 cc of each added to 90 cc of the basic medium described above. In one tube, which served as the control, 10 cc of normal saline were added to the basic medium. In each experiment, the basic medium was prepared originally in a single batch.

A few observations were also made on the effect of a saline extract of yeast when added to the basic medium in a final concentration of 0.2%. The yeast solution was made by dissolving 2 g of Difco yeast in 100 cc of normal saline and filtering the solution through a Seitz pad.

The different media were then tubed in 15 cc amounts, and heated at 57°C for 30 minutes. Subsequently, the tubes were inoculated with 0.5 cc of an actively growing culture of *L. icterohaemorrhagiae* in Noguchi's medium to which no growth factor had been added. In each experiment 5 or 6 different tubes of each medium were tested. Four separate experiments were run, although in one experiment cysteine and leucine were omitted. All tubes were incubated at 32°C, and were examined daily by the darkfield microscope.

Estimation of the growth in the tubes on a given day was based on a rough count of leptospira seen under the darkfield in 50 fields of a slide prepared from each tube. The approximate degree of growth was recorded as follows: No leptospira seen, —; 1-10 leptospira in all 50 fields, ±; 10-50 organisms in a total of 50 fields, +; an average of 3-20 leptospira per field, ++; 20-100 spirochetes per microscopic field, +++; leptospira in each field too numerous to count, ++++. All tubes designated as +++ or ++++ were considered to be satisfactory for use as antigen for the agglutination or complement fixation tests.

Results. In Table I are given the composite results of 4 different experiments. Each reading noted, with the exception of those for cysteine and leucine, is based on at least 20 tubes of a medium containing one added substance. The results show that the addition of nicotinic acid in a final concentration of 0.01% (10,000 gamma per 100 cc) materially accelerated the growth of leptospira, as compared with the standard media, so that only 3 or 4 days were required to obtain a maximum yield. Cysteine hydrochloride also accelerated the growth, though not as much as nicotinic acid. The results admittedly are based on rough estimates of the number of leptospira in each tube, but the differences were definite and consistent.

The tubes of media to which had been added glycine always showed poor growth, and few viable leptospira were demonstrable after 6 days' incubation at 32°C. The addition of valine, tyrosine, methionine, glutamine, and leucine seemed to have little enhancing effect on the growth of leptospira, and there were no marked differences between these tubes and the control tubes.

The effect of yeast, in the few observations made, was enhancing, though not as much so as nicotinic acid and cysteine. Zimmermann[1] also observed that the addition of a loopful of sterile culture of yeast to 1.0 cc of ordinary medium enhanced the growth of leptospira.

Summary. Nicotinic acid enhances the growth of leptospira when added to Noguchi semi-solid medium in a final concentration of 0.01%. Cysteine hydrochloride in the same concentration has a similar effect, though not as marked as nicotinic acid. Glycine seems to have a reverse effect, and may even act to suppress the growth of the organisms.

TABLE I.
Effect of Nicotinic Acid and Certain Amino Acids on Growth of Leptospira in Modified Noguchi Medium.

Substance added to basic medium	Degree of positivity* Days after inoculation						
	1	2	3	4	5	6	7
Nicotinic acid	+	++	+++	++++	++++	++++	++++
Cysteine hydrochloride*	±	+	+++	+++	++++	++++	++++
Tyrosine	—	±	+	++	+++	++++	++++
Valine	±	+	+	++	+++	++++	++++
Glutamine	±	+	++	+++	++++	++++	++++
Leucine*	±	+	++	+++	+++	++++	++++
Glycine	±	±	±	±	±	±	±
Methionine	±	+	++	+++	+++	++++	++++
Control	±	+	++	+++	+++	++++	++++

*Based on 3 experiments with a total of 18 tubes.

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[1] Zimmerman, E., *Zent. f. Bakt.*, 1936, **137**, 280. (Abstract—*Trop. Dis. Bull.*, 1937, **34**, 358.)

13207 P

Glucuronic Acid Production *in vitro* by Liver Slices from Rats Fed Dimethylaminoazobenzene (Butter Yellow).

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Surviving liver slices, when shaken in a saline phosphate medium (pH 7.4) in the Warburg apparatus, produce conjugated glucuronic acids in the presence of borneol, menthol or phenol.[1, 2] This production has been shown to be significantly increased in the presence of sodium lactate or sodium pyruvate.[1] Beuding and Ladewig[2] reported, furthermore, that the power of liver to conjugate borneol is reduced in severe phosphorus poisoning and is not affected in fatal chloroform poisoning.

The experiments here reported were undertaken to determine whether or not the capacity of rat livers to conjugate borneol with glucuronic acid in the presence of sodium lactate is impaired by the oral administration of dimethylaminoazobenzene, a compound which causes severe cirrhosis and malignant neoplasms of the liver of the rat.[3] The addition of 15% yeast to the basal diet has been shown to give complete protection against both types of liver change.[4, 5] Stevenson, Dobriner and Rhoads[6] have provided evidence that orally administered dimethylaminoazobenzene is split at the azo link in the course of its breakdown in the animal body, and that conjugated para-aminophenol is excreted in the urine. Para-aminophenol has been shown to be conjugated with glucuronic acid and sulfate *in vivo*.[7]

The method used to measure glucuronide formation *in vitro* was essentially that described by Lipschitz and Beuding.* [1] The assays of glucuronide in the urine were made by the method of Maughan, Evelyn and Browne.[8] The standard used was a sample of pure sodium pregnandiol glucuronide supplied through the courtesy of

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[1] Lipschitz, W., and Beuding, E., *J. Biol. Chem.*, 1939, **129**, 333.

[2] Beuding, E., and Ladewig, P., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 433.

[3] Kinosita, R.. *Trans. Jap. Path. Soc.*, 1937, **27**, 665.

[4] Ando, T., *Gann.*, 1938, **32**, 252.

[5] Sugiura, K., and Rhoads, C. P., *Cancer Research*, 1941, **1**, 1.

[6] Stevenson, E., Dobriner, K., and Rhoads, C. P., in press.

[7] Jaffee, M., *Z. f. Physiol., Chemie.*, 1888, **12**, 295.

* A Pfaltz & Bauer photoelectric colorimeter was used.

[8] Maughan, G. B., Evelyn, K. A., and Browne, J. S. L., *J. Biol. Chem.*, 1938, **126**, 567.

Dr. Browne. The urine used was collected over 3- to 4-day periods, from groups of 5 rats each. The diets used were the same as those previously described.[9]

The results of the experiments (Table I) indicate that the feeding of dimethylaminoazobenzene causes no impairment of the ability of liver slices to conjugate d,l borneol *in vitro*. Even livers which were markedly cirrhotic and contained hepatomas or cholangiomas gave normal or somewhat elevated values.

The results of the study of the urinary excretion of glucuronides by rats fed dimethylaminoazobenzene are summarized in Table II. Animals fed this compound excreted more glucuronides in the urine than did the rats fed the control diets. The amount of glucuronic acid found in the urine of rats which had ingested the chemical for over 100 days was found to be low, possibly due to the decreased intake of food containing the compound. The in-

TABLE I.
d,l Borneol Conjugation with Glucuronic Acid by Liver Slices *in Vitro* in the Presence of Sodium Lactate.

Group	No. of days on diet	No. of rat livers assayed	Mg glucuronic acid formed per g liver 90 min. Variation	Mg glucuronic acid formed per g liver 90 min. Avg value
Normal diet		8	.9-2.8	1.5
Basal diet	30-100	13	.8-3.4	2.0
Basal diet + dimethylaminoazobenzene	30-150	15	1.3-5.3	3.0
+ Dimethylaminoazobenzene + 15% Yeast	80-130	10	1.1-4.0	2.1
+ Dimethylaminoazobenzene + Non-protective Supplement	50-150	20	1.5-6.7	3.1

TABLE II.
Urinary Output of Glucuronides by Rats.

Diet	Days on diet (Interval studied)	No. Collections	Mg glucuronic acid per rat day Variations	Mg glucuronic acid per rat day Avg
Normal		10	1.1-10.0	5.2
Basal	1-52	10	3.8-15.0	6.1
Basal + 6 mg Dimethylaminoazobenzene (1)	1-40	12	8.8-22.2	12.9
+ 6 mg Dimethylaminoazobenzene (2)	1-32	10	9.2-18.9	13.2
+ 6 mg Dimethylaminoazobenzene (3)	[illegible]0-160	20	.8-5.8	3.2
+ Dimethylaminoazobenzene 15% Yeast	1-40	13	7.1-20.0	12.9
+ Dimethylaminoazobenzene + 15% Yeast	100-140	11	6.5-19.8	10.5

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[9] Kensler, C. J., Sugiura, K., and Rhoads, C. P., *Science*, 1940, **91**, 623.

gestion of food by rats in the early stages of dimethylaminoazobenzene feeding and of rats receiving a protective supplement is approximately 10 g per day per rat. Rats supplied the unsupplemented carcinogenic diet eat between 4 and 6 g per day when they have been on this diet for 100 days or more. Another contributing factor probably is that late in the experiment less aminophenol or other phenolic compounds are available for conjugation with glucuronic acid due to some metabolic change associated with the damage to the liver cells.

Conclusions. 1. The ability of rat liver slices to conjugate borneol with glucuronic acid *in vitro* is not impaired, even when marked cirrhosis and malignant degeneration of the liver are produced, by the feeding of dimethylaminoazobenzene. 2. The glucuronide excretion of rats fed dimethylaminoazobenzene is initially somewhat higher than in the control animals. However, after 100 days of dimethylaminoazobenzene feeding, the rate is less than 25% of that seen initially.

13208

Experimental Hypertension from Section of Moderator Nerves: Relationship to Presence of Kidney Tissue.*

CAROLINE BEDELL THOMAS. (Introduced by W. T. Longcope.)

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Hering,[1] Koch and Mies,[2] Heymans, Bouckaert and Regniers[3] and others have shown that section of both carotid sinus nerves and both aortic depressor nerves in experimental animals results in an immediate rise in blood pressure. Section of these 4 moderator nerves in dogs results in persistent hypertension which is essentially neurogenic in origin. Following total sympathectomy, this form of hypertension disappears, according to Heymans and Bouckaert.[4]

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* This work was aided by a grant from the Commonwealth Fund for the study of essential hypertension.

[1] Hering, H. E., *Die Karotissinusreflexe auf Herz und Gefasse,* Dresden, Th. Steinkopff, 1927.

[2] Koch, E., and Mies, H., *Krankheitsforschung,* 1929, **7**, 241.

[3] Heymans, C., Bouckaert, J. J., and Regniers, P., *Le Sinus carotidien,* Paris, 1933.

[4] Heymans, C., and Bouckaert, J. J., *Bull. de l'Acad. Royale de Med. de Belg.,* 1936, **6**, 42.

Grimson, Bouckaert and Heymans[5] produced hypertension in dogs completely sympathectomized save for the origin and distribution of the splanchnic nerve fibers to kidneys and adrenals by cutting the moderator nerves. Following denervation of the kidneys at the pedicle in 2 such animals, blood pressure fell to normal for 90 and 40 days of observation. They therefore postulated that the sustained neurogenic hypertension thus obtained had been of renal origin.

Heymans and Bouckaert[6] showed that the blood of dogs made hypertensive by moderator nerve section possessed a higher degree of vasopressor activity than the blood of normal dogs. This they attributed to an increased adrenalin content of the blood. Hermann, Jourdan, and Delrieu[7] demonstrated that in the dog made hypertensive by moderator nerve section, adrenalin is not the only substance present in the circulating blood responsible for the increase in peripheral vascular resistance. Page[8] has found that the blood of similar hypertensive animals showed vasoconstrictive effects when perfused through the rabbit's ear, which he attributes to increased amounts of angiotonin.

In view of the recent interest in the etiological relationship of the pressor substance angiotonin, isolated from kidney tissue, to other forms of experimental hypertension, and since the above mentioned reports bring up the possibility that angiotonin may play a rôle in the hypertension resulting from moderator nerve section as well, a series of acute experiments was carried out comparing the effect of moderator nerve section in normal animals and in animals in which both kidneys had been removed.

The 4 moderator nerves were sectioned in 19 dogs, anesthetized lightly either with ether or with chloralose. Ten were normal animals with kidneys intact. Nine had had both kidneys removed under ether anesthesia. Bilateral nephrectomy had been carried out 24 to 48 hours before the acute experiment in 7 instances; these animals were lively and in good condition. In one dog the kidneys were removed 4 days before, and in another dog immediately before section of the moderator nerves. Blood pressure was recorded directly from the femoral artery by means of a mercury manometer.

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[5] Grimson, K. S., Bouckaert, J. J., and Heymans, C., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 225.

[6] Heymans, C., and Bouckaert, J. J., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 94.

[7] Hermann, H., Jourdan, F., and Delrieu, A., *Arch. des Mal du Coeur,* 1939, **39**, 545.

[8] Page, Irvine, personal communication.

The 4 moderator nerves were cut in succession in varying order. The entire vago-sympathetic-depressor nerve trunk was severed on each side. The carotid sinus bifurcation was often removed at the time of carotid sinus nerve section.

Upon section of a single moderator nerve, arterial pressure usually rose abruptly for a minute or two, reaching a peak which was sustained for one to 3 minutes. Pressure then fell more gradually, generally attaining a new baseline somewhat above the original one in 5 or 10 minutes. The arterial blood pressure just preceding section of the first moderator nerve was compared in each experiment with the pressure at the peak of the response to section of the fourth moderator nerve. The total net rise (or fall) in arterial blood pressure from section of the 4 moderator nerves was thus observed. A net rise of 10% or more of the initial arterial pressure was observed in 9 of the 10 normal animals and in 6 of the 9 totally nephrectomized dogs. (Table I.) The mean initial pressure of these 9 normal dogs was 130 mm of mercury, while that of the 6 nephrectomized dogs was 98 mm of mercury. The mean rise in arterial pressure was 51 mm of mercury, or 38% of the initial pressure, in normal dogs, and 87 mm of mercury, or 87%, in nephrectomized dogs.

One normal animal showed a drop of 56 mm of mercury, or 34% of the original pressure of 188 mm of mercury. Two nephrectomized dogs also had a drop of pressure from initial pressures of 106 and 135 mm of mercury, amounting to 72 and 51 mm of mercury respectively, or to 68 and 38% of the initial pressure. All 3 of these experiments were technically faulty. In 2, well marked cyanosis denoting definite anoxemia was present and in the third, the arterial pressure fell spontaneously throughout the experiment, possibly from too large a dose of chloralose for that animal.

The single dog which was studied 4 days after nephrectomy seemed lethargic and weak before anesthetization, and the arterial pressure ranged from 62 to 67 mm of mercury throughout the experiment.

TABLE I.

		Initial arterial pressure in mm Hg			Rise in arterial pressure in mm Hg			Rise in arterial pressure in %		
	No.	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median
Normal dogs	9	74-197	130	120	14-100	51	42	13-62	38	42
Dog immediately after nephrectomy	1	—	100	100	—	82	82	—	82	82
Dogs 24-48 hr after nephrectomy	5	64-123	98	103	20-137	87	105	34-140	87	100

Section of the moderator nerves at no time produced any pressor or depressor response in this animal.

Summary. From the foregoing results it seems clear that in the dog, removal of both kidneys does not prevent the immediate appearance of a pressor response to moderator nerve section. The reflex response to such a procedure is at least equal to that seen in normal dogs and if anything, seems to be heightened. Chloralose and ether were each used as anesthetic agents in both groups of experiments. It so happened that one of the most marked pressor responses occurred after the use of each kind of anesthetic agent in both the normal and nephrectomized group of dogs. In view of the slightly different initial blood pressure levels in the two groups, as well as the difficulty in ascertaining the exact depth of anesthesia in each experiment, close quantitative comparison of the pressor responses in the two groups is not justified. It would seem, however, that circulating pressor substances arising from the kidney (*e. g.*, angiotonin) are not demonstrably important in the production of acute neurogenic hypertension.

13209

Comparative Study of Effect of Sulfadiazine and Sulfathiazole on *Staphylococcus aureus.*

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(Introduced by Chester S. Keefer.)

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Sulfadiazine has been found to be as effective as sulfapyridine and sulfanilamide in the treatment of experimental pneumococcal and streptococcal infections,[1, 2] and its administration to man causes relatively few toxic symptoms.[3, 4, 5] In staphylococcal infections

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[1] Feinstone, W. H., Williams, R. D., Wolff, R. T., Huntington, E., and Crossley, M. L., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 427.

[2] Robbin, R. O., Jr., and Winnek, P. S., *J. Am. Chem. Soc.*, 1940, **62**, 1999.

[3] Peterson, O. L., Strauss, E., Taylor, F. H. L., and Finland, M., *Am. J. Med. Sci.*, 1941, **201**, 351.

[4] Plummer, N., and Ensworth, H. K., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 734.

[5] Reinhold, J. G., Flippin, H. F., Schwartz, L., and Domm, A. H., *Am. J. Med. Sci.*, 1941, **201**, 106.

in mice, the percentage of survivals following treatment with sulfadiazine compared favorably with the number of survivals following the administration of sulfathiazole.[1] The results of comparing the relative effectiveness of sulfanilamide and sulfadiazine against hemolytic streptococci were reported in a previous paper.[6] At this time, we report the results of a comparative study of the effect of sulfadiazine and sulfathiazole on various pathogenic strains of *Staphylococcus aureus*.

Methods. The methods employed in this study have been described previously.[7, 8] In brief, the culture medium was defibrinated whole blood obtained from individuals without evidence of infection. The two drugs, sulfadiazine or sulfathiazole, were either added directly *in vitro* or were administered by mouth before withdrawal of the blood. Chemical analysis of all blood containing the drug was made.* Eight saline dilutions of an 18-hour broth culture of *Staphylococcus aureus* were made and 0.1 cc of each dilution was added to 0.5 cc of the defibrinated blood. The culture tubes were sealed and rotated in the incubator for 48 hours. The tubes were then examined for hemolysis; those cultures showing no hemolysis were opened and the contents plated out after proper dilutions had been made. The agar used in these plates contained para-amino benzoic acid.[9] Following incubation of the plates the colonies were counted.

Action of Sulfadiazine and Sulfathiazole When Added in vitro to Whole Blood. It was first necessary to determine what effect the addition of sulfadiazine *in vitro* had on the bactericidal power of blood obtained from normal individuals. Five milligrams of the powdered drug were added to 20 cc of defibrinated blood. After thorough mixing for one hour, various dilutions were made with drug-free blood as the diluent. Each dilution was then used as culture medium and the bactericidal action against the staphylococcus observed.

Fig. 1 shows the results obtained in 2 such experiments.

The addition of sulfadiazine to defibrinated blood caused an

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[6] Keefer, C. S., and Rammelkamp, C. H., to be published.

[7] Rammelkamp, C. H., and Keefer, C. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 664.

[8] Rammelkamp, C. H., and Keefer, C. S., *New Eng. J. Med.*, 1940, **223**, 877.

* Miss Lucy Perry made all chemical determinations. Only the non-acetylated amounts of the drugs are recorded in this paper. The sulfadiazine used in this study was supplied through the courtesy of Lederle Laboratories, Inc.

[9] Strauss, E., Dingle, J. H., and Finland, M., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 131.

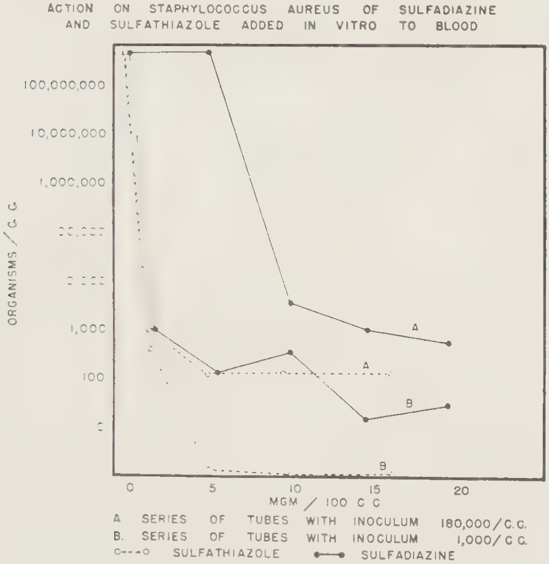

Fig. 1.

Points represent number of organisms present after 48 hours' incubation.

increase in the bactericidal power of the blood. When the original inoculum was greater than 1,000 organisms per cubic centimeter of blood, complete killing was not observed. This was found to be true even with concentrations of sulfadiazine as high as 20 mg per 100 cc. Blood containing less than 1,000 organisms per cubic centimeter usually exhibited some bacteriostatic action with concentrations as low as 2 mg per 100 cc. This action became bactericidal when levels of 5 to 10 mg per 100 cc were obtained. Concentrations above 10 mg per 100 cc produced only a slight increase in the bactericidal power.

We have shown previously[5] that the addition of sulfathiazole to whole blood caused bacteriostasis and an increased killing power against *Staphylococcus aureus*. In the present experiments sulfathiazole was somewhat more effective than sulfadiazine against pathogenic staphylococci when whole blood was used as the culture medium. In general, sulfathiazole completely killed inoculations of 10,000 organisms or less, which is about 10 times the bactericidal power observed following the addition of sulfadiazine to the media. A further point observed in these *in vitro* studies was that the action of sulfathiazole became manifest at low concentrations. When large inocula were used, bacteriostasis was more marked in the sulfathiazole-containing tubes.

Effect of Ingestion of Sulfadiazine on Bactericidal Power of

Blood. In this group of experiments, the blood was studied for bactericidal activity after the drug had been exhibited by mouth. A sample of blood was withdrawn before the administration of 4 or 5 g of sulfadiazine, and additional specimens were taken at intervals varying from 40 minutes to 4 hours after the ingestion of the drug. These samples were defibrinated and then used in the bactericidal tests.

Fig. 2 shows 2 typical responses observed in 30 experiments. It was clearly demonstrated that the administration of sulfadiazine by mouth to normal individuals increased the bactericidal effect of the blood against pathogenic staphylococci. We then studied more thoroughly the relation of the concentration of sulfadiazine in the blood to the bactericidal power observed. Fig. 3 illustrates the results obtained in one of these experiments. With a concentration of 1.3 to 1.6 mg per 100 cc no effect against pathogenic staphylococci was noted, even with a small inoculum of 20 organisms per cubic centimeter of blood. When the concentration reached 2.5 mg per 100 cc and the inoculum was less than 20,000 organisms per cubic centimeter, a definite bactericidal effect occurred. With concentrations up to 4.2 mg per 100 cc this action was even more marked. Levels of 4.8 and 5.2 mg per 100 cc caused some bacteriostatic action in blood containing 200,000 organisms per cc.

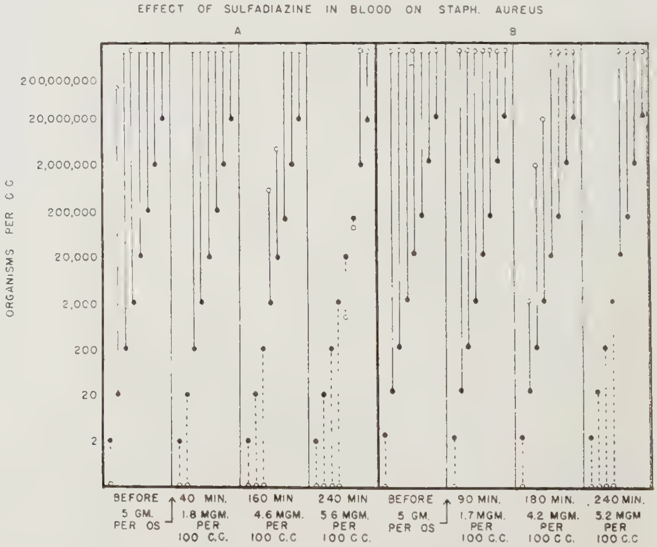

FIG. 2.

Solid dots represent original inoculum of *Staphylococcus aureus.* Circles represent number of organisms present after 48 hours' incubation. Solid lines indicate growth; interrupted lines indicate killing.

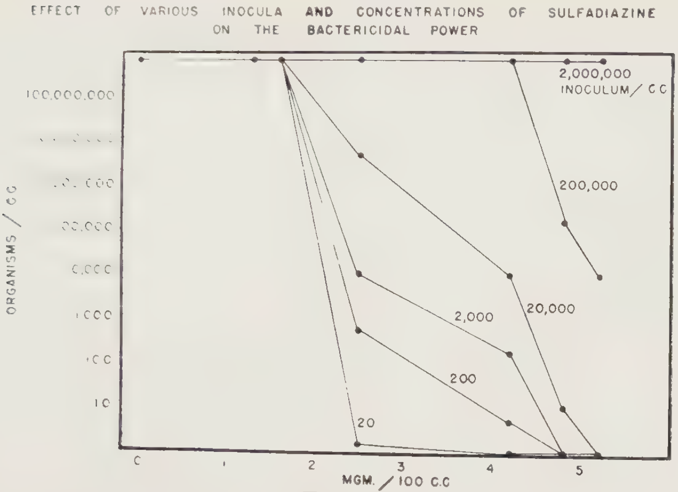

Fig. 3.

Solid dots represent number of organisms present after 48 hours' incubation. Lines connect cultures with the same original inoculum.

These studies showed, then, that concentrations of sulfadiazine of 4 to 5 mg per 100 cc are desirable in order to obtain effective bactericidal action.

Comparative Bactericidal Effect of Sulfadiazine and Sulfathiazole When Administered by Mouth. From the studies reported above, it was obvious that the addition of sulfathiazole to blood caused a greater increase in the bactericidal effect than sulfadiazine. Further, it was found that the maximal bactericidal action occurred at a lower concentration in the sulfathiazole-containing cultures. As a result of previous studies with sulfathiazole[8] it was our impression that sulfathiazole exerted a more marked action than sulfadiazine when these drugs were given by mouth.

The bactericidal power of these two drugs after oral administration was then examined and compared. Normal individuals were first given 4 g of sulfathiazole by mouth. A week later the same individual was given 4 g of sulfadiazine. In each instance blood was withdrawn before administration of the drug and several times thereafter. Bactericidal tests were carried out using an inoculum that was comparable in the two tests.

In Fig. 4 the results of 5 such comparative tests are recorded; these represent the typical results obtained in 9 such observations. It is at once evident that sulfathiazole given orally causes a greater increase than sulfadiazine in both the bactericidal and the bacteriostatic power of the blood. Occasionally the action of the two drugs is about equal, as is seen in test C. In test E the level of

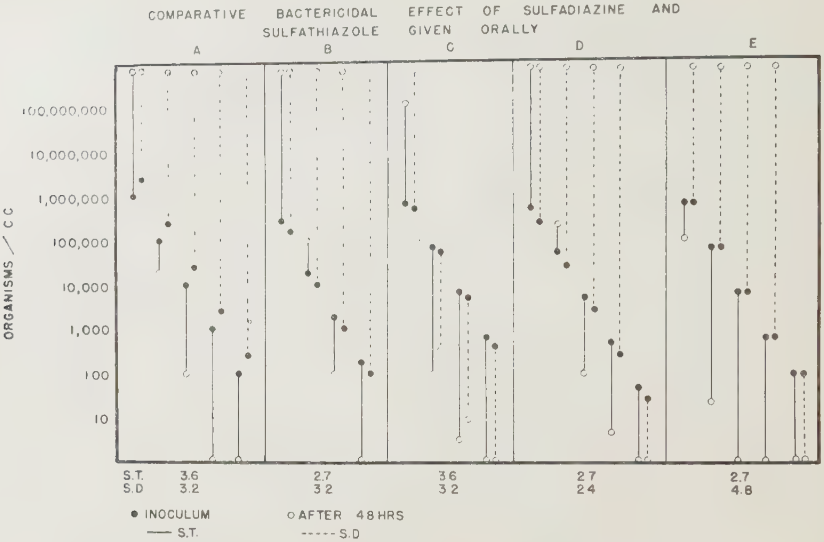

FIG. 4.

S.T.—sulfathiazole; S.D.—sulfadiazine.

sulfadiazine was 4.8 mg per 100 cc, and even in this individual a concentration of sulfathiazole of 2.7 mg per 100 cc was more effective against the staphylococcus.

Discussion. The present studies indicate that sulfadiazine increases the bactericidal power of normal blood against *Staphylococcus aureus,* both when added directly *in vitro* and when administered by mouth. The addition of the two drugs to similar samples of blood shows that sulfathiazole is somewhat superior to sulfadiazine against the staphylococcus. Further, the action of sulfathiazole becomes manifest at lower concentrations. Attempts to determine the level of sulfadiazine which produces maximal killing show that a concentration of 4 mg or above is usually necessary. Levels above 10 mg per 100 cc do not increase this action appreciably. This is in contrast to the action of sulfathiazole which produces maximal bactericidal action at a somewhat lower level.[7]

When the two drugs are administered orally, sulfathiazole invariably produces as great or greater an increase in the bactericidal action of the blood. This was true even though the concentration of sulfadiazine was twice that of sulfathiazole.

These results, then, indicate that sulfadiazine is not as effective in whole blood as sulfathiazole against the staphylococcus, even though high concentrations are obtained. It is felt, however, that sulfadiazine should prove useful in the treatment of patients with staphylococcal infections who do not tolerate sulfathiazole. It would also appear useful in the treatment of staphylococcal infections of the meninges inasmuch as sulfadiazine diffuses readily into the spinal fluid.

Conclusions. Sulfadiazine increases the bactericidal action of normal blood when added *in vitro* or when exhibited orally. Comparative tests show that sulfathiazole is somewhat superior to sulfadiazine in its effect on *Staphylococcus aureus.*

13210 P

Absorption and Metabolism of Estrogens. I. Estrogen Assay by Intrasplenic Injection.

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It has been stated that estrogens are metabolized by the liver of the rat and completely inactivated. The best evidence for this is the excellent work of Heller[1] on tissue slices incubated with various estrogens. Methods of indirect attack *in vivo* have consisted in attempting to study effects of endogenous or exogenous estrogens in animals in whom the liver had been poisoned.[2, 3] There have also been attempts to recover estrogens by hashing the whole animal after injection.[4] These have also led to the belief that estrogens are completely inactivated by the liver.

The present study of which this is a preliminary report was undertaken with the view of direct attack on this problem.

The estrogen in question was injected directly into the spleen in 0.05 cc of oil solution. The spleen was exposed through a mid-ventral incision and delivered to the outside; the site of entrance of the needle into the spleen was tied with an encircling silk ligature to prevent leakage of the oil. The injections were made into castrate females primed 14 days previously with 50 γ of estrone. Vaginal smears were taken with a moist, cotton tipped toothpick at 48, 60, and 72 hours after injection, and a fully cornified smear in any one or more of the three periods was considered as positive. These procedures were followed throughout except for a group of 10 animals on 100 γ of estrone, where it was necessary to use 0.1 cc of oil owing to the relative insolubility of the material.

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[1] Heller, C. G., *Endocrinology,* 1940, **26**, 619.

[2] Pincus, G., and Martin, D. W., *Endocrinology,* 1940, **27**, 838.

[3] Talbot, N. A., *Endocrinology,* 1939, **25**, 601.

[4] Zondek, B., and Sklow, J., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 276.

The intrasplenic unit is defined as that amount of estrogen required to produce positive smears in 50% of at least 10 rats treated as described. On the basis of this standard procedure the intrasplenic unit of alpha-estradiol* has been 50 γ, estrone about 70 γ, estradiol benzoate 24 γ, and estradiol dipropionate 5 γ. This study is being continued on these estrogens as well as stilboestrol, estriol, estradiol benzoate-butyrate, and estrone benzoate under various conditions.

These preliminary results lead to the following possible conclusions: (1) A large proportion (about 98%) of intrasplenically injected free estrogen is destroyed either in the liver or spleen. (2) The point of attack on estradiol is probably at the hydroxyl groups since partial protection of this estrogen is afforded by covering one hydroxyl group and greater protection by covering both hydroxyl groups. (3) The esters of estradiol are at least partially absorbed from the spleen as esters since the esterification afforded protection from destruction.

13211

Ineffectiveness of Thiamin Hydrochloride in Treating Late Weaning Paralysis of Vitamin E Deficient Rats.

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It has recently been reported by Holmes and Pigott[1] that orally administered thiamin hydrochloride is effective in curing the neuromuscular disorder which appears during late lactation in the offspring of rats receiving a restricted intake of vitamin E. These observations were based upon 3 litters in which paralysis appeared between the 20th and 22nd days of lactation. Ten offspring died from dystrophy or other causes before the remaining 10 afflicted rats were given vitamin B_1 treatment, supplemented by oral feeding of the E-deficient diet mixed with distilled water. Amounts of thiamin hydrochloride varying from 12 to 43 γ were said to have effected cures in the latter 10 rats. Since a certain number of such paralyzed rats can be expected to recover spontaneously[2] (a phenomenon noted by Holmes and Pigott in 3 other litters prior to the institution of B_1 therapy), it seemed desirable to investigate more fully the value of thiamin hydrochloride in the prevention and in the cure of this paralysis in suckling rats.

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* We would like to thank: Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products for supplies of alpha-estradiol (Ovocylin) and estradiol dipropionate (Di-ovocylin), Dr. Erwin Schwenk of the Schering Corporation for quantities of estradiol benzoate (Progynon-B), and Dr. Oliver Kamm of Parke, Davis and Company for estrone (Theelin).

[1] Holmes, A. D., and Pigott, M. G., *Am. J. Physiol.*, 1941, **132**, 211.

For this purpose 10 litters of paralyzed rats were used. The mothers of these litters, reared from the 14th day of life on a vitamin E-deficient diet (casein, 20; starch, 50; lard, 18; salts, 2.5; yeast, 7.5; cod liver oil, 2), were used in bioassay tests during their first pregnancy. A preliminary test was carried out on the litter of one rat given a low assay dose of rat liver. The 8 offspring showed paralysis at the 21st day of life, at which time daily administration of 0.5 mg of thiamin orally and 0.5 mg intraperitoneally was begun. Only one of the litter recovered, the others dying within 3 or 4 days.

The conditions employed for the production of the paralysis in the other 9 litters are summarized in Table I. It will be noted that the level of vitamin E dosage giving a positive response in 70% of assay tests permitted no lactation (group C), while 2 and 4 times this level resulted in satisfactory lactation (groups B and A). However, paralysis appeared in most of the offspring between the 17th and 20th days of life, which is somewhat earlier than in the litters studied by Holmes and Pigott. Approximately one-half of the members of each litter received 0.5 mg (1/20th cc of a 10 mg/cc solution) of thiamin hydrochloride orally, fed daily for a period of 4 to 5 days, the remaining members serving as untreated controls. When rats of either group appeared weak or paralyzed, they received a mixture of the E-deficient diet mixed with distilled water and fed by pipette at frequent intervals during each day and evening. The procedure was essentially the same as that used in the studies of Holmes and Pigott except for the fact that the amount of thiamin administered was greatly in excess of that found by them to be effective in curing paralysis. There is no reason to believe that the difference in the level of thiamin dosage in the two studies is of significance.

The first series of observations were made on the litters of group

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[2] Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 273.

TABLE I.
Source and Lactation Record of Paralyzed Litters.

					Lactation record			
Group	Assay dose vit. E conc. mg	No. of assay tests	Positive responses, %	Litters allowed to suckle	Total No. at birth	Died before onset of paralysis	Age at onset of paralysis, days	Age treatment begun, days
A	7.5	10	100	5	44	3	18-20	18-20
B	3.75	10	100	4	34	7	17-18	15
C	1.875	20	70	6	46	46*	—	—
D	0.937	10	0	—	—	—	—	—

*Died 2 to 4 days after birth.

A, in which treatment was initiated as soon as hyperflexion of the forehand or other early evidence of paralysis appeared. It will be noted (Table II) that the proportion of survivors was essentially the same in the treated and the untreated animals.

It is generally recognized that the earlier in lactation the neuromuscular disorder appears, the more severe are its symptoms and the lower is the incidence of spontaneous recovery. Since it was evident that the litters of group B would show earlier and more severe paralysis than those of group A, the treatment described above was begun 2 to 3 days before the first signs of paralysis appeared in any of the members (Table I). Paralysis was very marked and the frequency of recovery somewhat less than in the litters of group A. However, as in the case of the latter group (Table II), the proportion of recoveries was not significantly different in the treated and the untreated animals. Although the pipette feeding undoubtedly prolonged the survival time and facilitated recovery of the afflicted rats, the results give no indication that vitamin B_1 therapy alone exerted any beneficial effect.

TABLE II.
Deaths and Survivals in Thiamin-treated and in Untreated Paralyzed Rats (9 Litters).

			Experimental animals		
Group	No. of offspring	Killed*	Total	Died	Recovered
Treated					
A	22	2	20	8	12
B	14	0	14	6	8
A and B	36	2	34	14	20
Untreated					
A	19	10	9	3	6
B	13	5	8	4	4
A and B	32	14	17	7	10

*These animals were sacrificed for metabolic studies after moderate or advanced paralysis had appeared.

The E-deficient diet used in the studies of Holmes and Pigott, and in those reported here, should afford an ample supply of vitamin B_1 for the mother and offspring. It is also of interest to note that this neuromuscular disorder first came to the attention of Evans and Burr[2] when they had put into effect their observations that the vitamin B requirements of the rat are greatly increased during lactation. In fact, with increasingly larger proportions of yeast in their diets there was a corresponding increment in the number of young reared, in the average weight at weaning, and in the number of rats showing paralysis.

Conclusions. It is concluded that, contrary to the claims of other investigators,[1] oral administration of vitamin B_1 (thiamin hydrochloride) supplemented by force feeding is ineffective in preventing or in curing the paralysis in suckling rats attributable to an insufficiency of vitamin E.

13212

Failure to Find Sodium Pregnanediol Glucuronidate in Bull's Urine.

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The discovery by Marker, Wittle and Lawson[1] of large amounts of pregnanediols in bull's urine was made in extracts of the hydrolyzed urine. Also, though the paper of Marker and Hartman[2] appears to refer to presence of pregnanediol glucuronidate in pregnant cow's, pregnant mare's and bull's urine, examination of the papers they cite in this connection reveals no reference to actual demonstration of the presence of pregnanediols conjugated as glucuronidate.

The value given by Marker, *et al.*,[1] for the average amount per gallon of pregnanediol 3α, 20α in bull's urine is 100 mg. Dividing this by Venning's[3] factor 0.597, one finds that this is equivalent to

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[1] Marker, R. E., Wittle, E. L., and Lawson, E. J., *J. Am. Chem. Soc.*, 1938, **60**, 2931.

[2] Marker, R. E., and Hartman, C. G., *J. Biol. Chem.*, 1940, **133**, 529.

[3] Venning, E. H., *J. Biol. Chem.*, 1937, **119**, 473; 1938, **126**, 595.

167 mg sodium pregnanediol glucuronidate per gallon, an amount well within the range of estimation by the Venning[3] procedure. We therefore applied this technic to urine samples obtained from the bladders of 4 freshly slaughtered bulls and 2 steers, the volumes being 1360, 840, 320, 300, 480, and 1060 cc respectively. Each urine was promptly extracted with butanol, and run through the usual method as for human urine.[3] The extracted urine, the extracted 0.1 N NaOH and washings, were pooled and set aside for hydrolysis.

The steer urine appeared to be greatly dilute compared to that of the bulls.

On evaporation of the second butanol extracts under reduced pressure, white material was noted, but on the usual precipitation from 5 cc water and 95 cc anhydrous acetone no precipitate was obtained except in one case, where on reprecipitation and weighing, 3.7 mg of material melting instantaneously at 167° was found. The acetone solutions were evaporated and those of the 4 bulls pooled. These combined residues (from 2820 cc of bull's urine) weighed 1.2 g and contained an easily sublimed white material, but none of the easily recognizable sodium pregnanediol glucuronidate.

Summary. No sodium pregnanediol glucuronidate was found in urine obtained from the bladders of slaughtered bulls and steers by the method of Venning.[3] The volumes of urine were judged sufficient for demonstration of this substance if it were present in the amount suggested by Marker's results for pregnanediol 3α, 20α from hydrolyzed bull's urine.

13213

Bactericidal Property of Blood Serum of Male Rabbits Treated with Urinary Estrogens.

S. N. Tsao. (Introduced by C. N. Frazier.)

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There is clinical and experimental evidence of a definite relationship between hormones and immunity to infection. The early work on the effect of the suprarenal glands on resistance to disease was reviewed by Perla and Marmorston.[1, 2] The effect of insulin on

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[1] Perla, D., and Marmorston, J., *Arch. Path.*, 1933, **16**, 379.

[2] Perla, D., Proc. Soc. Exp. Biol. and Med., 1934-35, **32**, 797.

infection has also attracted much attention.[3] Recently, Cope and Kapnick[4] reported changes in complement-concentration and in response to vaccinial infection following alterations in thyroid, adrenal, and pituitary function in the rabbit and dog.

Pregnant mare's serum has been shown to neutralize *in vitro* the virus of poliomyelitis,[5] and Aycock[6] has found that treatment with estrogenic hormone protected castrated monkeys against the same disease. Sprunt and his co-workers[7] observed the favorable effect of estrogenic and gonadotropic hormones on vaccinial infection in rabbits, and Weinstein[8] has recently demonstrated the prophylactic value of certain hormonal preparations, such as those of the anterior pituitary gland and parathyroid, against anthrax in mice. He failed, however, to find any effect of estrin on this infection. Working on adult rabbits treated with estrogenic hormone, the same author[9] demonstrated an increase in the circulating agglutinin for *E. coli* and hemolysin for sheep cells.

There have also been numerous studies of the relation of sexual maturity to immunological reactions.[10]

The relationship between sex-hormones and immunity to syphilis was suggested by the observation of Frazier, Mu, and Hu[11] in this laboratory. It has been shown that estrogenic hormones extracted from the urine of pregnant women enhance the resistance to syphilitic infection of male rabbits feminized by the action of such substances.[12]

The purpose of the present paper is to report a study of the effect of estrogenic hormone on the natural antibody-level of adult male rabbits as measured by the bactericidal property of their blood serum on *E. typhi.*

Material and Method. A total of 72 adult albino rabbits were used, of which 60 were male and 12 female. Of the 60 male rabbits, 29 were treated 6 times a week with 40 R.U. of estrogen (equivalent approximately to 102 international units of estrone) in olive oil for 300 days or more. This extract was prepared in our laboratory according to the technic previously described.[12] Fifteen of the rabbits were treated with the same amount of olive oil as control. The other 16, together with the female rabbits, were not treated. The male rabbits were not castrated.

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[3] Marble, A., White, H. J., and Fernald, A. T., *J. Clin. Invest.*, 1938, **17**, 423.

[4] Cope, O., and Kapnick, I., *Endocrinology*, 1940, **27**, 533.

[5] Jungeblat, C. W., and Engle, E. T., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 879.

[6] Aycock, W. L., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 573.

[7] Sprunt, D. H., McDearman, S., and Raper, J., *J. Exp. Med.*, 1938, **67**, 159.

[8] Weinstein, L., *Yale J. Biol. and Med.*, 1938-39, **11**, 369.

[9] Weinstein, L., *Yale J. Biol. and Med.*, 1938-39, **11**, 169.

[10] Baumgartner, L., *Yale J. Biol. and Med.*, 1933-34, **6**, 403.

[11] Frazier, C. N., Mu, J. W., and Hu, C. K., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 65.

[12] Frazier, C. N., and Hu, C. K., *Endocrinology*, 1941, **28**, 283.

Blood was withdrawn from the ear under aseptic conditions. The serum was separated and used without dilution or heating. The organisms used, *E. typhi,* were grown on meat-infusion agar for 18 hours at 37°C. At the end of that time they were washed off with saline, making an even suspension of 320 million organisms per cc, from which serial dilutions were made; 0.1 cc of the serum was added to an equal amount of the different dilutions of the bacterial suspension. A serum-control without organisms was included in each sample. The tubes were well shaken and incubated at 37°C for 2 hours. At the end of this time, 3 to 5 cc of blood-digest broth with 1% dextrose and litmus indicator were added to each tube, and the mixtures were again incubated at 37°C for 48 hours. The final result was observed at the end of the second incubation. The lowest dilution of the suspension in which there was no evidence of growth was taken as the endpoint.

One control determination was made on each animal before treatment, and a second determination after 6 days of treatment. After these two determinations, bleeding was done at different intervals varying from 25 to 300 days or more after the beginning of treatment. Some of the animals were tested more than once.

Results. The results are recorded in Table I. The figures represent the number of organisms destroyed and are based on the mean values of the total number of animals in each group, indicated by *N,* together with their standard deviations. The significance of the differences between the various groups was estimated by the statistical *t*. *P* represents the probability of the difference being due to chance.

Discussion. From the table, one can see readily that there was a marked increase of the bactericidal power of the blood serum after one week of treatment with estrogen. As the treatment was prolonged, there was an increase of bactericidal power as compared with the status of the normal male rabbits before treatment. The reason for the inclusion of the results on the group treated for 25 days with those of 300 days was that the mean value of the 12 determinations in the former group failed to show any significant variation from the figures obtained in the latter group. That the increase in bactericidal power was not due to the olive oil in which

TABLE I.
Effect of Estrogenic Treatment on Bactericidal Power of Blood Serum of Rabbits in Terms of Millions of Organisms Killed.

Groups of comparison					
	Normal male	Treated male	Difference	t	P
6 days of estrogenic treatment	1.287 ± 0.29 $N = 17$	7.529 ± 1.34 $N = 17$	6.242	8.568	0.001
25 to 300 days of estrogenic treatment	0.907 ± 0.13 $N = 60$	1.743 ± 0.29 $N^* = 39$	0.836	2.970	0.01-0.001
Oil treatment	0.858 ± 0.27 $N = 15$	0.967 ± 0.09 $N = 15$	0.109	0.3445	0.8-0.9
	Normal male	Normal female			
No treatment	0.907 ± 0.13 $N = 60$	0.667 ± 0.17 $N = 12$	0.240	0.7758	0.4-0.5

*The number of tests among 27 animals.

the estrogenic substance was dissolved is shown by the lack of significant difference with results before and after treatment with the oil alone. The bactericidal property of the blood from normal male rabbits did not differ significantly from that of normal female rabbits.

Conclusion. The administration of urinary estrogens increased the bactericidal property of the blood serum of adult male rabbits against *E. typhi.* This increase was greater shortly after the beginning of treatment. With prolonged treatment, the increase was less apparent but still significant up to the end of the experiment, that is to say, after 300 days of treatment. Whether this was a direct response to the estrogen administered or secondary to other changes in the body induced by hormonal action needs further investigation.

13214

In vitro Effect of Sulfanilamide, Sulfapyridine and Sulfathiazole on *Corynebacterium diphtheriae.*

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The purpose of the present investigation is to determine the effect *in vitro* of sulfanilamide, sulfapyridine and sulfathiazole on *C. diphtheriae.* Observations are also made as to how long it takes for effective action, and on the effect of sulfanilamide, sulfapyridine and sulfathiazole on various serological types of Mitis organisms. Nitti, Phillips and Bovet reported that sulfanilamide retards the development of diphtheria bacilli in culture.[1] In the same year, Rosa treated diphtheria carriers by applying the drug locally but failed in this attempt.[2]

Methods and Materials. One to 1,000 dilutions of sulfanilamide and sulfapyridine were prepared from a 1% sulfanilamide solution and Soludagenan (1 g/3 cc) respectively, and standardized by means of the Klett-Summerson photo-electric colorimeter.* One to 1,000 dilution of sulfathiazole was prepared by heating aqueous solution containing a definite weight of the chemical. Final dilutions of 1:10,000 or 10 mg % were prepared by adding 0.2 cc of stock solution to 1.8 cc of liver digest broth, pH 7.6, containing 5% horse serum. All strains of *C. diphtheriae* were local strains isolated from the throats or noses of patients or carriers and were virulent to Chinese hamsters. One-tenth cc of 1:1,000 dilution of a 24-hour broth culture was used as the inoculum of the first 2 experiments, and a loopful of undiluted 24-hour cultures used in the third one. Survival of organisms was determined by growth on blood agar plates.

Experiment 1. The results of the experiment are summarized in Table I. It represents one of the 3 identical experiments. Under similar conditions, sulfathiazole seems to have exerted a very definite bactericidal action on representative strains of all the 3 cultural types of *C. diphtheriae,* while the other 2 were less effective on one Mitis strain than on strains of the other cultural types.

Experiment 2. In this experiment, frequent subcultures were made at definite intervals on tubes containing given amounts of organisms and 1:10,000 dilutions of sulfathiazole. It was found that the bactericidal activity of the chemical on all the same 3

TABLE I.
Number of *C. diphtheriæ* in Broth Containing 10 mg % Sulfanilamide, Sulfapyridine and Sulfathiazole Before and After 48 Hours of Incubation.

	Mitis (3458)		Intermedius (Park 8)		Gravis (776)	
Chemical	Before	After	Before	After	Before	After
Sulfanilamide	10^5	29x10^9	5x10^5	100	6x10^5	160
Sulfapyridine	10^5	26x10^7	5x10^5	1080	6x10^5	0
Sulfathiazole	10^5	0	5x10^5	0	6x10^5	0
Control	10^5	18x10^9	5x10^5	55x10^9	6x10^5	82x10^9

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[1] Nitti, F., Phillips, M., and Bovet, D., *Compt. rend. Soc. de biol.*, 1939, **131**, 70.

[2] Rosa, A., *Bull. Soc. Ital. Biol. sper.*, 1939, **14**, 630.

* All determinations were kindly performed by Dr. S. Y. Pan.

strains did not start until at least 4-8 hours of incubation have elapsed.

Experiment 3. Forty-three strains of *C. diphtheriae* belonging to 6 different serological types[3] were tested for their susceptibility to 1:10,000 dilutions of sulfanilamide, sulfapyridine and sulfathiazole. One loopful of 24-hour cultures was employed as inoculum, which is much larger than those previously used. The results are presented in Table II. It was observed that different strains of the same serological type reacted differently to the various chemicals, and that sulfathiazole is by far the most efficient bactericidal agent of the three. All those strains which were susceptible to sulfanilamide were invariably susceptible to sulfapyridine and sulfathiazole. A few of the strains which were found to be resistant to sulfathiazole, when retested with a smaller inoculum (0.1 cc of 1:1,000 dilution of a 24-hour culture), turned out to be susceptible also to the same chemical, a finding which is in agreement with those reported by others on other bacteria.

Comment. The present experiments have confirmed and extended the observations made by Nitti and his coworkers. Not only were the growth of these organisms retarded, marked bactericidal action of several common sulfanilamide compounds, on *C. diphtheriae* with appropriate inoculum was observed. Of the 3 chemicals tested, sulfathiazole was active on the largest number of strains studied, and sulfanilamide least. That it requires 4-8 hours of contact between the chemicals and the organisms before their bactericidal action can be appreciated might explain the failure in the treatment of diphtheria carriers by local application as reported by Rosa.

TABLE II.
Susceptibility of Various Serological Types of *C. diphtheriæ* to 10 mg % of Sulfanilamide, Sulfapyridine and Sulfathiazole.

Serological types	No. of strains tested	No. of strains susceptible to		
		Sulfanilamide	Sulfapyridine	Sulfathiazole
II	2	2	2	2
III	12	6	7	8
IV	2	1	1	2
V	13	1	4	6
VI	1	1	1	1
VIII	13	6	6	9
Total	43	17	21	28

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[3] Huang, C. H., in press.

13215

Atrophy of Germinal Epithelium of the Rabbit's Testis Induced by Roentgen Rays.

C. K. Hu and C. N. Frazier.

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In an attempt to produce atrophy of the germinal epithelium of the rabbit's testis, a series of adult animals were subjected to roentgen irradiation of the scrotal region. It was hoped, at the same time, to preserve, if possible, the functional integrity of the glandular cells of the testis.

This report describes the technic which was found adequate to induce complete and uniform degeneration of the entire spermatogenic series without appreciably suppressing the hormonal activity of the testis for such periods of time as the animals were observed.

Material and Method. Altogether 24 male rabbits of an albino breed, from 6-13 months of age, were treated with roentgen rays. Both testes were irradiated at the same time through the anterior scrotal wall. The adjacent parts of the body were protected with lead rubber sheets.

The X-ray machine employed was of a type producing a half wave current through 2 valve rectifiers. It was equipped with an oil immersed tube unit possessing an inherent filtration equivalent to approximately 1 mm of aluminum. The tube was operated on a current of 3 ma at a peak voltage of 140 kv. The target skin distance was 18 cm. A filter of 1 mm of aluminum was interposed, making a total filtration equivalent to approximately 2 mm of aluminum. With this setting, the machine delivered 126 r per minute when fields not over 100 sq cm were exposed. The erythema skin dose (E.S.D.) was 517 r.

Each animal was given 6 treatments at intervals of 3 days. The time of exposure was 3 minutes at each treatment. This made a total of 2,268 r (4.4 E.S.D.) for each animal over a period of 15 days.

The rabbits were examined once a week, and the period of observation extended from 2 to 6 months after the first treatment. Six animals were killed for histological study of the testes, 2-3 months after the first irradiation. Three of these had been subjected to both vasectomy and irradiation, while the other 3 had received only irradiation. The changes observed, however, were common to all animals.

Results. The first effect of the rays was shown by the testes becoming flabby in consistency. This change was detected as early as 9 days after the first treatment in 1 animal, 13 days in another, 16-18 in 7, and 21-24 days in the remainder.

A gradual and progressive decrease in size of the testes was next observed. In the course of about a month subsequent to the change in consistency, or 6-7 weeks after the first irradiation, the testes were reduced to about half their original size, or less. Thereafter, no further reduction in size was detected.

None of the animals showed any gross alteration in the size of the penis or of the scrotum, in a period of 6 months. The psycho-sexual behavior of the animals was not affected by treatment.

Untoward Reactions. A transient and moderate edema developed in the scrotum of one animal and in the prepuce of another, presumably as a result of the irradiations. Other reactions were not encountered. The general condition of the animals remained good.

Organ Weights. The testes showed a reduction in weight to less than one-half of the values obtained in another series of animals not irradiated.[1] The secondary sex organs, however, showed no appreciable decrease in weight. As a matter of fact, the seminal vesicles showed a tendency to increase in weight (Table I).

Histological Study. Histological examination of the testes revealed no difference between those animals that received X-rays alone and those that underwent vasectomy in addition to the roentgen irradiations. The specimens were obtained from 30 to 86 days after the first irradiation. The first specimen was obtained at biopsy. The sections were stained with hematoxylin and eosin.

Within 30 days of the first irradiation, or 15 days after the last, there was a marked atrophy of the germinal epithelium, and the seminiferous tubules had decreased in size (Fig. 1). However, one could still find a few spermatogonia. A 58-day specimen showed

TABLE I.
Weights of Sex Organs of Normal, Orchidectomized, and Irradiated Rabbits.

Group	Testes		Seminal vesicles		Bulbourethral gland	
	N	Mean $\pm \sigma_m$ mg/kg	N	Mean $\pm \sigma_m$ mg/kg	N	Mean $\pm \sigma_m$ mg/kg
Normal	17	1002.4 ± 84.0	18	526.9 ± 64.7	17	189.5 ± 22.5
Orchidectomized	—	—	8	128.7 ± 11.7	8	66.2 ± 10.2
Irradiated	5	437.7 ± 59.7	6	697.0 ± 39.0	5	157.9 ± 6.0

N = Number of animals.

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[1] Frazier, C. N., and Hu, C. K., *Endocrinol.*, 1941, **28**, 283.

practically complete disappearance of the germinal cells, leaving behind a single layer of Sertoli cells. There appeared to be a few atypical spermatogonia. Specimens of 78-86 days showed, in addition to the changes stated above, a varying degree of fibrosis (Fig. 2). This process involved the interstitial tissue as well as the remnants of the tubules. All the changes were remarkably uniform throughout the sections examined.

In some instances, there appeared to be a relative increase in the amount of interstitial tissue together with an increase in the number of Leydig cells. This seemed to be due to the marked decrease in the size of the seminiferous tubules, and the resulting condensation of the remaining interstitial tissues.

Discussion. Using the technic described we obtained a rather prompt, uniform and clear-cut destruction of the germinal epithelium of the seminiferous tubules in the adult rabbit, a species of animal known to be relatively resistant to roentgen rays. We share

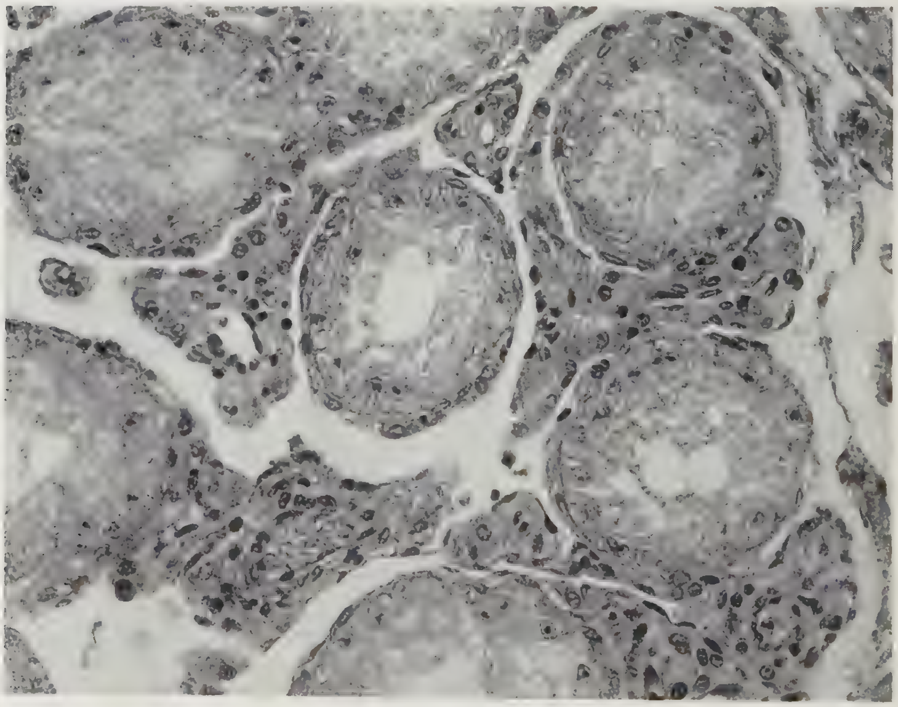

FIG. 1.
Section of testis 30 days after the first roentgen irradiation, showing marked atrophy of germinal epithelium. Only Sartoli cells remain in the tubules. × 295.

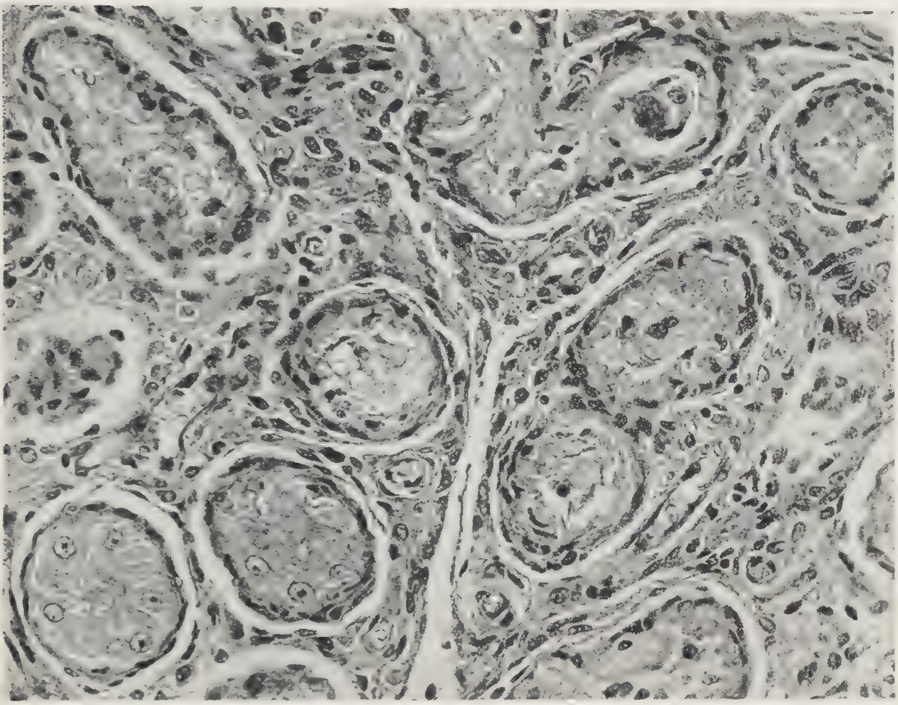

FIG. 2.

Section of testis removed 80 days after the first roentgen irradiation, without vasectomy. Note the general fibrotic appearance, complete atrophy of the germinal epithelium and reduction in size of the seminiferous tubules. × 295.

the opinion with Ferroux, Regaud and Samssonow,[2] who have successfully used a similar technic, that the effects of the X-rays seem to be permanent.

No changes in the interstitial tissue of any physiological significance were demonstrated. The animals retained their sexual power in spite of the loss in fertility. Furthermore, the penis, scrotum and the secondary sex organs did not show atrophic changes within the period of observation.

The structural relationship of the sexual organs to the function of the testis was shown in 8 adult rabbits subjected to castration. In these there occurred a shrinkage of the scrotal sacs and complete atrophy of the glans penis within 3 months following removal of both testes. There was also a considerable decrease in weight of the secondary sex organs (Table I).

The histological changes observed in the animals subjected to both vasectomy and irradiation were practically the same as those found in animals receiving only irradiation. Furthermore, in another series of animals in which the influence of vasectomy was studied, the degeneration in the germinal epithelium was neither as uniform nor as complete as that resulting from the roentgen rays alone.

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[2] Ferroux, R., Regaud, Cl., et Samssonow, C., *Compt. rend. Soc. de biol.*, 1938, **128**, 173.

The data on hand seem to indicate that the roentgen rays have a selective action on the germinal epithelium of the testis.

Summary. Testes of adult rabbits subjected to a series of exposures to X-rays aggregating 2,268 r (4.4 E.S.D.) over a period of 15 days showed complete atrophy of the germinal epithelium. The secretory function of the testes, as judged by the maintenance of the normal size of the penis and scrotal sacs, and by the normal psycho-sexual behavior of the animals, was not affected as long as 6 months following irradiation.

13216 P

Immunity and Experimental Relapsing Fever in the Chinese Hamster (*Cricetulus griseus*).

Y. P. CHEN. (Introduced by Samuel H. Zia.)

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While the problem of immunity in relapsing fever of man and of laboratory animals has been extensively investigated, no definite conclusion has been reached owing to the contradictory findings. However, it is generally agreed that immunity following an attack of relapsing fever is of short duration but occasionally a long-lasting immunity may occur which has usually been attributed to the persistence of residual infection, notably in the brain.[1-4] In connection with our studies on the course and chemotherapy of experimental relapsing-fever infection by a California strain in Chinese hamsters,[5] opportunity arose for a careful examination of certain phases of immunity in this infection with special attention to its relation to

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[1] Hindle, E., *A System of Bacteriology*, London, His Majesty's Stationery Office, 1931, **8**, 146.

[2] Hindle, E., *Trop. Dis. Bull.*, 1935, **32**, 309.

[3] Coleman, G. E., *J. Infect. Dis.*, 1934, **54**, 1.

[4] Beck, M. D., *J. Infect. Dis.*, 1937, **60**, 64.

[5] In press.

residual brain-infection and to the influence of treatment in the development of immunity.

Twenty hamsters, both treated and untreated, which survived 20 to 43 days after spirochetes had disappeared from the peripheral blood, were used. These animals, 4 of which were treated with neoarsphenamine,* 6 with trisodarsen,† and 4 untreated, were reinoculated with blood from one infected hamster, the amount inoculated being double that used in the original inoculation. The remaining 6 animals served as controls and were not reinfected. Following inoculation, blood smears were made daily on all animals during the period of observation.

Results. Among the neoarsphenamine-treated group, 2 showed complete and the other 2 partial resistance. In the latter cases spirochetes were found for only 1 or 2 days after a prolonged incubationary period of 5 to 8 days. Of the 6 treated with trisodarsen, 5 were completely protected, while the other showed partial immunity. Of the untreated animals, one showed partial immunity while the rest were completely resistant. No relapses were seen in any of these animals. As expected, the control animals, which were not reinfected, did not show spirochetes in the peripheral blood during the course of observation. It may be noted that of the 4 hamsters which showed spirochetemia during the second infection, 3 belonged to the group infected with mouse- rather than hamster-blood at the first inoculation. This finding seems to indicate that the immunity conferred by the first infection is apparently stronger against reinfection with homologous blood than with heterologous blood.

For detecting residual infection in the brain of both partially and completely immune animals and those not reinfected, about half of those that survived 17 days after the second inoculation were sacrificed for examination. Sections of the brain were stained with Steiner's method.[6] All the animals so examined showed large number of spirochetes in the brain. Spirochetes could be found in the brain of infected hamsters, treated or untreated, as late as 60 days after the spirochetes had disappeared from the peripheral blood. At the same time, it was found that all the animals remained at least partially immune for as long as 43 days after the apparent cure of the first infection. This correlation suggests that residual infection plays an important part in the production of immunity to spirochetal infection in Chinese hamsters.

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* Purchased locally; prepared by the Abbott Laboratories, North Chicago, Illinois, U. S. A.; Lot No. 905L004.

† Supplied through the courtesy of the Abbott Laboratories, North Chicago, Illinois, U. S. A.; Lot No. 811L073.

[6] Quoted by Spielmeyer, von W., *Technik der Mikroskopischen Untersuchung des Nervensystem*, p. 160, Julius Springer, Berlin, 1930.

Comment. (1) Chinese hamsters apparently recovered from infection with a California strain of relapsing-fever spirochetes were resistant to subsequent infection. Treatment did not seem to influence this immunity to any appreciable degree. (2) A suggested correlation between residual infection in the brain and immunity appears in the present experiment.

Grateful acknowledgment is due Dr. Samuel H. Zia of the Department of Bacteriology and Immunology, P.U.M.C., for his helpful advice and suggestions, and Dr. Y. K. Hsu of the Division of Neurology and Psychiatry for the staining and examination of brain sections.

13217

Effect of Diethylstilbestrol Dipropionate on Mammary Development and Lactation.

SHEPPARD M. WALKER AND ALLAN J. STANLEY. (Introduced by Albert E. Casey.)

From the Department of Zoology, Louisiana State University.

The prolonged effect of the dipropionate ester of diethylstilbestrol has been shown to maintain rats in estrus 50 days after a single 10 γ injection.[1] The influence of this estrogen upon mammary development and lactation was studied on heifers and rats. Two Jersey heifers, one a castrate at 12 months of age and the other a sterile 3-year-old, were used. Twelve castrate and 14 intact lactating rats were employed to further check the results obtained in the heifers.

The castrate heifer was injected over a period of 9 months with a total of 1560 mg of diethylstilbestrol dipropionate* and 530 mg testosterone propionate† at the end of which time milking was begun. During the first half of the injection period only the estro-

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[1] Dodds, E. C., Goldberg, L., Lawson, W., and Robinson, R., *Nature,* 1938, **142**, 211.

* Stilbestryl dipropionate contributed by The Lakeside Laboratories, Inc., Milwaukee, Wisconsin.

† Oreton contributed by Schering Corporation, Bloomfield, New Jersey, and Perandren contributed by Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

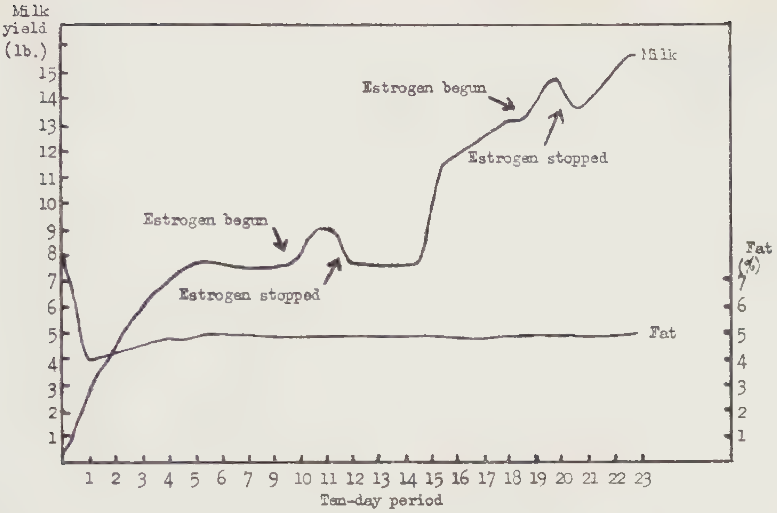

Fig. 1.

Lactation record of a castrate grade Jersey heifer injected with diethylstilbestrol dipropionate.

gen was used. The udder developed slowly under this treatment and reached about one-fourth of its present size. During the latter half of the 9-month period testosterone propionate was added to the estrogen in the proportion of 4 parts estrogen to 1 of androgen. The udder responded by more than doubling its size. All treatment was stopped at the end of 9 months and daily milking was begun. Small quantities of milk were obtained during the first few milkings but the yield increased rapidly and was more than 3 pounds at the end of 10 days. The lactation record of this animal is shown in Fig. 1.

Twice during the lactation period additional injections of diethylstilbestrol dipropionate alone were given. In the first instance 110 mg were given in alternate daily doses of 10 mg each. At the beginning of this series of injections milking had been in progress for 100 days and the daily yield was 8 pounds. The yield immediately increased to 9½ pounds but after 50 mg had been given a sudden decline began which continued during the remainder of the 21-day injection period and carried the production to 7½ pounds. This level was maintained for the next 30 days when the yield increased rapidly and reached a new high level of 13 pounds. The second series of injections was begun on the 180th day of lactation after the 13-pound level had been maintained for 10 days. Sixty mg were given in 10 mg doses on alternate days. The production immediately rose to 15 pounds and again receded to 13 pounds at which time the injections were stopped. During the following 20 days the yield rose to a new high of 16 pounds per day. It is to be noted that this animal was castrated 10 months before any injections were given and that no progesterone was ever given. It is also worthy of note that the production rose immediately after injections were made during the lactation period. No inhibition ensued until a much higher titer was established. The period of time following the "hump" in the production curve after estrogen administration during which the inhibition continued (recovery time) seems roughly proportional to the dosage. The constancy of the fat percentage during the increased yield seems good evidence that new parenchyma was developed during the recovery period. This would then represent a true growth of the mammary gland and not merely an "enrichment effect" of Folley.[2, 3] The immediate response to the estrogen when the titer is known to be low and the rise occurring at the end of the recovery time when the titer is once again low show that the new parenchyma develops under these titers. Flaccidity of the udder was always noted to accompany the establishment of an injuriously high titer in both lactating and non-lactating glands.

The sterile heifer was injected with a total of 350 mg of diethylstilbestrol dipropionate over a 90-day period and milking was then begun. During the first 30 days of lactation the yield reached 3.7 pounds per day. At this time alternate daily 5 mg doses of diethylstilbestrol dipropionate were begun and continued for 43 days until a total of 85 mg had been injected. During this 43-day period the milk yield rose to 8.1 pounds. At this time the injections were stopped and within the next 17 days the milk production increased to 14 pounds per day. During the 43-day injection period the udder increased markedly in size. It is to be noted in this animal that the titer of estrogen was slowly built up so that no actual inhibition occurred until the very end of the period. Upon cessation of estrogen administration the titer again became low with a consequent increase in udder growth and milk production.

Diethylstilbestrol dipropionate was given in 0.2 mg and 1.0 mg doses to castrate and intact lactating rats, whose litters had been reduced to 6 at parturition. Injections were given daily from the second to the twentieth day after parturition. The average growth rate of the young was used as a measure of the rate of lactation.

The average growth rate of the young of all treated mothers exceeded that of the controls through the second day of the injec-

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[2] Folley, S. J., *Lancet*, 1941, **240**, 6124.

[3] Folley, S. J., and Young, F. G., *Lancet*, 1941, **240**, 6134.

tion period. After the second day of treatment, the young whose mothers were left intact gained weight less rapidly than the sucklings of non-castrate controls. On the contrary, the rapid growth rate was continued for 6 days in the young of the castrate mothers which were given 1.0 mg, and for 12 days in the litters of castrate mothers which were given 0.2 mg doses. The suppression of lactation was much more pronounced in intact than in castrate animals. The ovaries, adrenals, and pituitary of those animals that were injected showed a pronounced increase in weight over those of the control mothers.

The premature opening of the vagina which occurred at 12 days after birth in most of the treated litters indicates that large amounts of the estrogen were secreted with the milk and also that this hormone is not readily attacked by any of the enzymes of the rat.

Conclusions. 1. Progestin is not necessary for complete mammary development in cattle. 2. Estrogens will induce mammary development and copious milk secretion in cattle without injection of prolactin. 3. The turgidity of the udder may be used as an indicator of the secretory activity of the non-lactating gland. 4. The titer of estrogen determines its effect on the mammary gland. A low titer induces proliferation of the parenchyma and secretion of milk; a high titer suppresses lactation and brings on involution of the mammary gland.

13218

Renal Carbonic Anhydrase.

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The presence of carbonic anhydrase in the mammalian gastric mucosa has been reported and discussed by Davenport and Fisher[1] and by Davenport.[2, 3] Although the rôle of carbonic anhydrase in the gastric secretion of hydrochloric acid is not yet directly established the presence of this enzyme in high concentration in the cells of a tissue primarily concerned with the secretion of ions is of particular interest. One of the chief functions of the kidney is the tubular transfer of ions, and if carbonic anhydrase in tissues is generally part of the chemical mechanism performing such transfer it might be expected that carbonic anhydrase is present in kidney tissue. This expectation is realized in the experiments described in this paper. The kidneys of cats, dogs and rats have been found to contain concentrations of carbonic anhydrase of the same order of magnitude as have been found in blood and gastric mucosa.

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* Sterling Fellow, 1940-41.

[1] Davenport, H. W., and Fisher, R. B., *J. Physiol.*, 1939, **94**, 16P.

[2] Davenport, H. W., *J. Physiol.*, 1940, **97**, 32.

[3] Davenport, H. W., *Am. J. Physiol.*, 1940, **128**, 725.

Methods. The animals were anesthetized with ether, and the abdominal aorta was exposed. A cannula was inserted into the aorta distal to the kidneys, and after the aorta proximal to the kidneys had been clamped off 0.9% sodium chloride solution was forced through the kidney blood vessels by gravity. Perfusion was continued until the fluid flowing from the cut renal veins was free of blood. The kidneys were then excised, and slices of them were weighed on a torsion balance, ground in a glass mortar and extracted with water.

Carbonic anhydrase was determined by the method of Meldrum and Roughton.[4] The apparatus was used at 0°C and at atmospheric pressure. The activity was calculated and expressed as enzyme units (E) according to the method of Meldrum and Roughton, but no correction was applied to bring the calculated activity to 15°C. The enzyme unit used in this work is therefore 2 to 3 times smaller than that of Meldrum and Roughton.

Results. Carbonic anhydrase was found in the kidney extracts. The extracts catalyzed the hydration and dehydration of carbon dioxide. The addition of the extracts to the buffer used in the enzyme determination did not change the pH of the buffer as measured by a glass electrode. The end points of the catalyzed and uncatalysed reactions were the same. The activity of the extracts was destroyed by 30 sec boiling, by heating to 65°C for 30 min and by acid and alkali. The activity was inhibited by 0.001 M HCN, by 1 mg % sulfanilamide and by 0.003 M NaSCN.

The activity of the extracts was not caused by the presence of carbonic anhydrase derived from red blood cells remaining in the kidney blood vessels. The perfusion of the kidneys was complete, and no areas of infarction were found. Histological sections of the perfused cat and dog kidneys were made, and a careful microscopic search of their blood vessels revealed no red cells. Cat blood contains about 3.7 E per cmm,[2] and dog blood contains from 3.1 to 7.4 E per cmm.[3] Slices of cat kidney contained up to 3.8 E per mg and of dog kidney up to 2.2 E per mg. Had the presence of blood carbonic anhydrase been responsible for the observed activity a large fraction of the slices would have been made up of red cells, and the hemoglobin associated with carbonic anhydrase in the red cells would have been easily detectable. The perfused kidneys were yellow-brown in color and showed no signs of containing any hemoglobin. The extracts ranged from opalescent white to yellow in color while samples of blood from the same animals diluted to the same carbonic anhydrase activity were bright red and obviously contained at least 50 times as much hemoglobin. When tested with the benzidine reaction the kidney extracts were either negative or very faintly positive while the blood samples diluted to the same enzyme concentration were strongly positive. Consequently it can be concluded that carbonic anhydrase is present in the kidney tissue itself.

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[4] Meldrum, N. U., and Roughton, F. J. W., *J. Physiol.*, 1933, **80**, 113.

Fourteen samples of cat kidney cortex obtained from 4 kidneys contained 2.6 ± 0.6 E per mg wet weight, and 13 samples of dog kidney cortex contained 1.8 ± 0.3 E per mg wet weight. There is no carbonic anhydrase in the medulla of either the cat or dog kidney. When rat kidneys were sliced no distinction could be made between medulla and cortex. Ten samples of rat kidney obtained from 6 kidneys contained 1.1 ± 0.4 E per mg wet weight. If there is no carbonic anhydrase in the rat kidney medulla the concentration in the cortex is probably still higher.

Discussion. Davenport[2] showed that the concentration of carbonic anhydrase in aqueous extracts of the gastric mucosa is of the same order of magnitude as that found in the kidney cortex. The concentration is lower than that in red cells, but when it had been shown that the enzyme is confined to the cells of the surface epithelium and to the parietal cells it could be calculated that the concentration of the enzyme in the cells is several times higher than in red blood cells. In this study it has not been demonstrated that carbonic anhydrase is localized in any particular type of cell in the kidney cortex. If it can be shown that the enzyme is in one type of cell and not in another it may be found that the enzyme is actually present in the cells in very high concentration.

The presence of carbonic anhydrase in the kidney makes possible the study of the functions of this enzyme in an organ more accessible and more readily controlled than is the gastric mucosa. At present the rôle of carbonic anhydrase in the kidney is only the subject of speculation, but the effects on the kidney of sulfanilamide, a powerful inhibitor of carbonic anhydrase, offer a promising line of attack on the problem.

Summary. Carbonic anhydrase is present in significant concentrations in the cortex of cat, dog and rat kidneys.

13219

A Method for Detecting in Human Serum Protective Bodies Against Hemolytic Streptococci.*

H. W. DIEFENDORF. (Introduced by Grover F. Powers.)

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The antigenic structure of the Group A hemolytic streptococci has been greatly clarified by the important work of Lancefield. Antistreptococcal rabbit serum has been used extensively in these studies for determining antigen-antibody relationships. There has been as yet no similar analysis of the antibodies in human serum from streptococcal disease. The passive protection of mice would be a useful approach to such a study. Neufeld[1] used this technic in human streptococcal disease but failed to obtain protection with the one scarlatinal serum tested. The work of Dochez, Avery, and Lancefield,[2] and of Hirst and Lancefield[3] indicates that in using mouse-protection it may be necessary to employ strains of streptococci which kill with 10^{-8} cc of culture. Unfortunately, freshly isolated strains of streptococci do not often kill in dilutions greater than 10^{-4} cc.[4, 5] Recently, however, the use of 50% endpoints in biological titrations has become more and more popular. This method of analysis has been made simple and practicable by Reed and Muench.[6] Accordingly, it appeared worth while to attempt to

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* Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

[1] Neufeld, F., *Z. f. Hyg.*, 1903, **44**, 161.

[2] Dochez, A. R., Avery, O. T., and Lancefield, R. C., *J. Exp. Med.*, 1919, **30**, 179.

[3] Hirst, G. K., and Lancefield, R. C., *J. Exp. Med.*, 1939, **69**, 425.

[4] Wadsworth, A., and Coffey, J. M., *J. Immunol.*, 1935, **29**, 505.

[5] Wu, J. P., *J. Immunol.*, 1941, **40**, 179.

[6] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

use fresh human strains of streptococci of only moderate virulence in protection-tests, carried out in such a way as to make use of the 50% endpoint method.

Materials and Methods. 1. *Media.* The basic culture medium was beef-heart infusion-broth containing 1% neopeptone. Blood broth was prepared by adding 0.1 cc of defibrinated rabbit blood to 5 cc of broth.

2. *Organisms.* Hemolytic streptococci were obtained from children by throat cultures on blood-agar plates. Typical hemolytic colonies were picked and the organisms identified by stained smear, bile-insolubility, and the presence of soluble hemolysin for rabbit erythrocytes. The organisms were cultured in blood broth, and were used within one month after isolation for the virulence- and protection-tests.

The number of streptococci injected into mice was determined by pour-plates; 10^{-7} cc of the 5-hour cultures contained 30 to 70 organisms in the 14 protection-tests.

3. *Virulence-tests.* White mice weighing 18-22 g were used for both virulence- and protection-tests. Preceding the actual protection-experiment a virulence-test was done with the patient's organism. A young culture was prepared by adding 0.1 cc of an 18-hour blood-broth culture to 5 cc of blood broth and incubating for 5 hours. This culture was diluted serially in broth in multiples of ten. Five-tenths cc of each dilution was inoculated intraäbdominally into one mouse, and the approximate time of death recorded. The 3 highest dilutions of culture which killed mice were used for the protection-test.

4. *Sera.* Blood was drawn by venapuncture and the serum stored in the icebox without preservative. Serum from the 10 scarlet-fever patients was taken during the latter part of the third week of the disease, from the 4 rheumatic-fever patients approximately one month after the onset of the attack under study. Control serum was obtained from 3 normal persons without a history of recent streptococcal infection. Control serum from one individual was used for 12 tests, and 2 other persons contributed serum for the 2 remaining tests.

5. *Protection-tests.* Undiluted serum was inoculated intra-abdominally 12 hours before the dose of streptococci. For the first few tests 0.5 cc serum was used. Later it was found that 0.25 cc gave protection also, so for economy this dosage was used for the remaining tests.

Hirst and Lancefield[3] showed that passive immunity with anti-streptococcal rabbit serum was type-specific. Consequently the serum from each patient was tested against the organism isolated from him, in order to take advantage of type-specific action if it were present.

Eight mice, 4 receiving patient's serum and 4 control serum, were used for each of the 3 dilutions of streptococci. Each mouse was injected intraäbdominally with 0.5 cc of the diluted culture of the patient's organism, prepared in the same manner as for the virulence-tests. Observations were terminated at the end of 4 days. In each test at least one mouse dying before 4 days was necropsied and heart-blood cultures were made on blood-agar plates. A pure growth of hemolytic streptococci was obtained in every case.

A few protection-tests were carried out by inoculating the organisms intracerebrally following the procedure suggested by Huang and Sia,[7] but the results were so irregular that the method was not used for further work.

Results. The results of the protection-tests are shown in Table I. For each dilution of hemolytic streptococci, the mice injected with the patient's serum and those receiving normal serum are represented side by side, and for simplicity will be referred to subsequently as 'serum-injected' and 'control' mice respectively. The numerator

TABLE I.
Protection of Mice Against Hemolytic Streptococci with Human Convalescent Serum.

		Dilutions of Hem. Strep.									
		10^{-1}		10^{-2}		10^{-3}		10^{-4}		10^{-5}	
		Injected Mice									
Patients	Disease	Serum	Control	Serum	Control	Serum	Control	Serum	Control	Serum	Control
W.B.	Scarlet fever					4/4	1/4	4/4	0/4	4/4	4/4
J.M.	” ”			0/4	0/4	2/4	0/4	3/4	3/4		
J.P.	” ”	1/4	0/4	4/4	0/4	4/4	1/4				
D.W.	” ”	0/4	0/4	0/4	0/4	4/4	0/4				
R.F.	” ”			4/4	0/4	3/4	1/4	4/4	3/4		
R.W.	” ”	0/4	0/4	1/4	0/4	3/4	0/4				
G.V.	” ”			0/4	0/4	2/4	1/4	4/4	4/4		
M.N.	” ”			1/4	0/4	3/4	0/4	4/4	2/4		
P.R.	” ”			0/4	0/4	0/4	0/4	2/4	2/4		
A.J.	” ”			0/4	0/4	1/4	1/4	3/4	2/4		
B.B.	Rheum. fever	0/4	0/4	4/4	0/4	4/4	1/4				
B.H.	” ”					0/4	0/4	3/4	0/4	3/4	1/4
J.M.	” ”	0/4	0/4	0/4	0/4	3/4	0/4				
W.T.	” ”			2/4	0/4	3/4	1/4	4/4	4/4		

Serum—Mice receiving patients' serum.
Control—Mice receiving normal serum.
Fraction—No. mice survived 4 days/No. injected.

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[7] Huang, C. H., and Sia, R. H. P., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 87.

of each fraction signifies the number of mice surviving 4 days, and the denominator, the number of mice injected. It will be noted that the surviving mice in most cases were distributed over 2, and sometimes over all 3 dilutions, which shows how difficult it is to choose the MLD and to express protection in terms of it.

However, the results can be analyzed quite readily by using the method of 50% endpoints recommended by Reed and Muench.[6] By calculating for each test the dosage of organisms required to kill half the serum-injected mice, and by comparing this figure with the dosage killing half the controls, some indication of the protective power of convalescent serum is obtained.

The results of the analyses of the 14 tests are listed in Table II. The calculated dilutions killing half the serum-injected and control animals (50% endpoints) are given for each test. The last figure on the right, the 'factor', is the number obtained by dividing the dilution killing half the serum-animals by that killing half the controls. For example, in the test on the serum from patient W.B. in Table II, 1/100 cc of 5-hour culture killed half the serum-animals, but the control animals were killed by 1/40,000 cc. In other words, 400 times as many organisms were required to kill the serum-injected mice as the controls. In one instance, the killing dosage for the serum-animals was 500 times that for the corresponding controls. In another case the 50% endpoints for the 2 groups

TABLE II.
Demonstration of Protection with Human Convalescent Serum Using the 50% Endpoint Method.

		Dilutions of Hem. Strep.*		
Patients	Disease	Serum-injected mice	Control mice	Factor
W.B.	Scarlet fever	1:100	1:40,000	400.0
J.M.	” ”	1:2,000	1:7,000	3.5
J.P.	” ”	1:30	1:4,000	133.3
D.W.	” ”	1:500	1:10,000	20.0
R.F.	” ”	1:10	1:5,000	500.0
R.W.	” ”	1:500	1:10,000	20.0
G.V.	” ”	1:1,000	1:3,000	3.0
M.N.	” ”	1:500	1:10,000	20.0
P.R.	” ”	1:10,000	1:10,000	1.0
A.J.	” ”	1:5,000	1:8,000	1.6
B.B.	Rheum. fever	1:50	1:4,000	80.0
B.H.	” ”	1:8,500	1:400,000	47.1
J.M.	” ”	1:700	1:10,000	14.3
W.T.	” ”	1:200	1:3,000	15.0

Serum-injected mice—Mice receiving patients' serum.
Control mice—Mice receiving normal serum.
*Dilution of broth culture of hemolytic streptococci calculated to give 50% endpoint.

coincided, but in no case was the protective power of the control serum greater than that of serum from patients.

For purposes of convenience, a purely arbitrary criterion for protection is used: sera with a 'factor' below 10 are considered non-protective; a 'factor' above 10 is assumed to indicate that protection was demonstrated. By this criterion, of the 10 scarlet-fever sera, 6 were protective and 4 were not. All 4 of the rheumatic-fever sera gave protection. Grouping all the tests together, protection was demonstrated in 10 out of 14 sera.

It may be added that the totalled results were subjected to the *chi*-square test and found to be statistically significant.

Discussion. Lancefield has described 2 type-specific antigens, M and T, in Group A hemolytic streptococci.[8] She showed that the M-anti-M complex was largely responsible for mouse-protection, and that the T-anti-T complex was not concerned with protection. However, antisera prepared with a glossy strain and devoid of anti-M detectable by the precipitin-test did give protection against 10 to 100 MLD of the homologous matt organisms. This observation led Mrs. Lancefield to suggest the possibility of another type-specific protective body, which is as yet poorly defined; this is another reason why the serological analysis of human streptococcal blood may prove valuable.

Summary. A method has been described to demonstrate that serum of patients convalescing from rheumatic fever and scarlet fever will protect mice against hemolytic streptococci.

13220

Assay of Sodium-Retaining Substances.*

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Sodium retention is an important function of the adrenal gland. The efficacy of adrenal preparations depends in part on this property. Methods for the assay of sodium-retaining substances have been

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[8] Lancefield, R. C., *J. Exp. Med.*, 1940, **71**, 521.

* Aided by grants from the National Research Council Committee on Research in Endocrinology and the Comly Fund of the Ohio State University.

described.[1, 2] In this paper we give the results of 3 years' experience with the method of Hartman and Spoor.[2] We are proposing certain modifications including the use of desoxycorticosterone as a standard.

Methods. The preparation and care of the animals have been described previously.[2] Animals showing evidence of kidney damage were discarded. The diet was adjusted so that the weight of each animal was maintained at an essentially constant level. For the average 10 to 12 kg dog the diet consisted of 250 g of beef heart, 70 g of Purina Dog Chow, 1 yeast pill and 2 g of sodium chloride daily. Cod liver oil capsules were given once a week. They were allowed water *ad lib.* except on test days. The animals were fed at 11 p.m., 9 hours before the beginning of a test period. They were catheterized at the beginning and end of the experiment and kept in metabolism cages during the 6-hour test. At the beginning of the test they were given 100 cc of distilled water by stomach tube.

All injections were given subcutaneously in order to prolong the effect and to avoid the development of refractoriness.[3] The material to be injected was dissolved either in peanut oil or 10% ethyl alcohol. Standard solutions of desoxycorticosterone were prepared and the response of each test animal was determined following the injection of a given dose (usually 0.6 to 0.8 mg was used). If the dose injected gave less than 35% or more than 65% retention of sodium the test was repeated using a modified dose. If the unknown to be tested was dissolved in alcohol, then the standardization dose of desoxycorticosterone was given in alcohol; if in oil, both materials were dissolved in oil. From time to time, injections of 10% alcohol or oil alone were made in order to determine any possible effect that the solvent might have on electrolyte excretion.

Sodium was determined by the method of Butler and Tuthill,[4] potassium by Consolazio and Talbott's modification[5] of the Shohl and Bennett method and chloride by the Volhard-Harvey method.[6]

The normal electrolyte excretion (control level) of the dogs was determined for the 6-hour test period. Control tests were run daily until it was established that the control level was essentially

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[1] Harrop, G. A., and Thorn, G. W., *J. Exp. Med.*, 1937, **65**, 757.

[2] Hartman, F. A., and Spoor, H. J., *Endocrinology*, 1940, **26**, 871.

[3] Hartman, F. A., Lewis, L. A., and McConnell, K. P., *Endocrinology*, 1939, **24**, 197.

[4] Butler, A. M., and Tuthill, E. J., *J. Biol. Chem.*, 1931, **93**, 171.

[5] Consolazio, W. V., and Talbott, J. H., *J. Biol. Chem.*, 1938, **126**, 55.

[6] Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, **2**, 834, Williams and Wilkins, Baltimore, 1932.

constant. A control was then run the day before each test day. At least one day was allowed to intervene between an injection and the following control period. The percentage of sodium retention was calculated using the average of the 2 controls immediately preceding the test as the normal level.

Results. In our experience to date we have used twelve dogs for sodium retention assay. These were selected from 48 healthy animals. As high as 100 assays have been made in a single dog during 2.5 years. Assays could not be made during hot weather because the results were so variable. With air conditioned quarters this might be possible.

The sodium retention of different dogs when injected with desoxycorticosterone is summarized in Table I. So far we have had but one dog (No. 48) which gave little or no response to the usual dose of desoxycorticosterone. The response in different animals varies somewhat. However, the important consideration is whether the same animal gives a constant response to the same dose. Some individuals are better than others in this respect. On this account they were used most frequently for assay. The responses of Dogs 32 and 42 were reasonably constant when it was realized that the

TABLE I.
Sodium Retention Caused by Desoxycorticosterone in Different Dogs.

Dog No.	Weight, kg	Date	Dose, mg	Retention %	Retention per mg	
37	7.8	3/31/40	.60 intrav.	41	68	
44	8.0	1/30	.60 ”	61	100	
45	8.2	4/2	.60 ”	40	67	
5	7.0	1/30	.75 ”	51	69	
32	10.0	12/2/39	.75 ”	49	66	
	10.0	1/12/40	.75 ”	49	66	
	9.1	10/11	.80 subcu.	43	54	
	9.9	12/3	.80 ”	53	66	Avg 63
42	10.2	1/16/40	.75 ”	46	61	
	10.5	10/11	.80 ”	51	64	
	10.2	12/6	.80 ”	60	73	
	9.9	4/18/41	.75 ”	51	69	Avg 66
31	7.5	1/30/40	.80 ”	45	56	
	7.1	10/11	.80 ”	35	44	
	7.0	12/3	.80 ”	50	62	Avg 54
48	18.7	2/28/41	.80 ”	0		
		3/4	1.00 ”	19	19	

first injections of desoxycorticosterone were given many months before the final injection.

In specific sodium retention no other substances except water, sufficient to maintain isotonicity, should be retained. Any additional retention can be attributed to an anti-diuretic effect. If, for example, potassium is also retained the latter effect may be suspected.

We define the unit for sodium retention as one-tenth of that amount of material which will cause the same sodium retention as 0.7 mg of desoxycorticosterone in the same dog. This quantity of desoxycorticosterone will cause retention within the sensitive range (35 to 65%) in most dogs. Calculation can be made by the following formula:

$$\text{No. of units} = \frac{\%\text{ retention by unknown}}{\begin{array}{c}\%\text{ retention by 0.7 mg}\\ \text{desoxycorticosterone}\end{array}} \times 10$$

If a different amount of desoxycorticosterone is used the results are calculated on a basis of 0.7 mg.

For example, 5 cc of an extract gave 40% retention. In the same dog 0.6 mg of desoxycorticosterone gave 36% retention or 0.7 mg should have given 42% retention.

$$\text{No. of units} = \frac{40}{42} \times 10 = 9.5$$

or 1.9 units per cc.

Table II gives examples of the assay of different extracts. If a

TABLE II.
Assay of Different Extracts.

Extract	Dog No.	% Na retention	Desoxycorticosterone (0.7 mg) % Na retention	Units per cc
Commercial A	5	45	48	1.8
	37	46	48	2.0
	42	50	45	2.2
” B	31	37	38	2.0
	37	32	48	1.4
Experimental I	31	37	38	1.95
	32	33	44	1.50
” II	5	49	48	2.04
	31	35	38	1.84
	42	46	46	2.0
” III	32	33	44	.74
	42	31	46	.67

larger number of animals were used for each preparation the assay could be improved.

Discussion. We prefer female dogs because they are easier to catheterize and one is more certain of complete evacuation of the bladder. However, they can not be used when they become pseudo pregnant. The sodium retention produced by adrenal extract or its fractions is essentially reproducible in any one dog. When compared with desoxycorticosterone the results elicited by a given preparation in different dogs may be similar. Why an occasional animal departs from this is not understood. There seems to be little correlation between size of animal or period of year and response.

It is realized that the sodium retention alone may not give the entire picture of the effect of a fraction on the electrolyte balance. It is felt, however, that assays are satisfactory if there is no decrease in the potassium excretion from the control level and if the water retention is not more than that necessary to maintain isotonicity. Under these conditions the retention produced by the extract may be considered specific sodium retention.

We believe that the method described can be employed to assay various substances which cause sodium retention. All commercial adrenal extracts should be assayed for this property because these sodium-retaining substances reduce the amount of extract required.[7]

We are indebted to Dr. R. D. Shaner of Roche-Organon Company for the desoxycorticosterone, and to Dr. G. F. Cartland of Upjohn and Company and Dr. David Klein of Wilson and Company for adrenal extracts.

Summary. A method is described for the assay of sodium-retaining substances in which desoxycorticosterone is employed as a standard. The sodium retention produced by the unknown is compared with that of desoxycorticosterone in a normal dog on a constant diet, over a 6-hour period.

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[7] Hartman, F. A., Lewis, L. A., Gabriel, J. E., Spoor, H. J., and Brownell, K. A., *Endocrinology,* 1940, **27**, 287.

13221

Gastric Secretion in Dogs Treated with Histamine Antagonist, Thymoxyethyldiethylamine.

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The phenol ether, thymoxyethyldiethylamine (929F), has been designated a sympathicolytic drug since it diminishes or reverses the action of adrenalin.[1, 2] This drug has been reported to antagonize the stimulating action of histamine upon isolated uterine and ileal muscle of the guinea pig.[3-6] Furthermore, pre-treatment with 929F prevents severe bronchiospasm and death in guinea pigs resulting from histamine liberated during anaphylactic shock or from the intravenous injection of several minimal lethal doses of histamine.[3-5, 7-9]

These facts suggest that 929F may antagonize the powerful stimulating action of histamine upon the gastric glands which secrete hydrochloric acid. If such an antagonism were demonstrated, 929F could be used to elucidate the question as to whether histamine plays any rôle in the secretory response of the stomach to food. Experiments have therefore been made to determine the effect of 929F upon the gastric secretion induced by injecting histamine.

Methods. At operation, 3 dogs weighing 7 to 10 kg were provided with Heidenhain pouches. After recovery from the operation the dogs were trained to stand quietly to facilitate the collection of gastric juice from the pouches. All dogs were maintained on a diet of canned meat and were fasted 15 to 24 hours before experimental periods.

The volume and acid content of the gastric juice collected during a 2-hour period following the subcutaneous administration of 0.5

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[1] Levy, J., and Ditz, E., *Compt. rend. Soc. biol.*, 1933, **114**, 971.

[2] Bovet, D., and Maderni, P., *Ibid.*, 1933, **114**, 980.

[3] Staub, M., *Ann. Inst. Pasteur*, 1939, **63**, 400.

[4] Staub, M., *Ibid.*, 1939, **63**, 485.

[5] Rosenthal, S. R., and Brown, M. L., *J. Immunol.*, 1940, **38**, 259.

[6] McVicar, G. A., and McHenry, E. W., *Canadian Med. Assn. J.* (Proc. Canadian Physiol. Soc.), 1941, **44**, 80.

[7] Bovet, D., and Staub, M., *Compt. rend. Soc. biol.*, 1937, **124**, 547.

[8] Staub, M., and Bovet, D., *Ibid.*, 1937, **125**, 818.

[9] Minard, D., and Rosenthal, S. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 237.

mg of histamine diphosphate constituted the control data. After a rest period of 1.5 to 2 hours, a subcutaneous injection of 10 mg of 929F* per kg of body weight was made. Thirty minutes later, the same amount of histamine as that used in the control period was administered subcutaneously. Each sample of gastric juice collected during control and experimental periods was titrated *in toto* for free and total acid with the use of Töpfer's reagent and phenolphthalein, respectively, as indicators.

The total volume of gastric juice and total quantity of free and total hydrochloric acid secreted following histamine injections in dogs treated with 929F were compared with the output obtained from animals earlier the same day when histamine alone was administered. Variations in gastric secretion during experimental periods are expressed as percentage increase or decrease from the control values. This so-called "double histamine test" is an established method of determining the influence of various factors on gastric secretion. We have also made control experiments to determine whether the gastric secretion following the second histamine injection, when the animals were not pre-treated with 929F, was comparable with the secretion obtained following the first histamine injection made earlier the same day.

Results. Following an injection of histamine the secretory response during 10 experiments on 3 dogs treated with 929F was found to be not less, *but more* than the secretory output in the same number of control experiments (Table I). The augmentation of free hydrochloric acid after treatment with 929F amounted to an increase of 73.9% over the average control value. Values for total acid were higher than, but paralleled, the values for free hydrochloric acid. The average total volume of gastric juice secreted by the animals treated with 929F represented an increase of 56% over the control value. Since the increase in volume is less than the increase in the quantity of free and total hydrochloric acid it is apparent that the concentration of hydrochloric acid in the gastric juice secreted after treatment with 929F was also increased. In dogs treated with 929F, hypersecretion and hyperacidity were consistent findings in each of the 10 experiments, although the degree was variable (free acid, 29 to 180%).

The adequacy of the control data was verified by employing the double histamine test 9 times on the 3 dogs without injecting 929F previous to the second histamine administration. The average total

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* A 5.0% solution of 2-isopropyl-5-methylphenoxyethyldiethylamine hydrochloride (M.P. 130-131°C).

TABLE I.
Augmentation of Gastric Secretion After Pre-treatment with 929F.*

Time in min.	Volume (cc)		Total acid (mg HCl)		Free acid (mg HCl)	
	Histamine Control	929F + Histamine	Histamine Control	929F + Histamine	Histamine Control	929F + Histamine
15	1.7	3.9	5.93	13.01	4.18	11.88
30	6.1	8.5	22.16	33.99	20.15	30.29
45	4.2	5.9	15.15	25.34	14.27	24.40
60	1.2	2.1	3.97	8.46	3.44	8.01
75	.8	1.2	1.90	4.10	1.85	3.73
90	.6	.9	1.04	2.67	.85	2.17
105	.5	.8	1.28	1.52	.62	.94
120	.1	.2	.07	.33	.04	.09
Total output	15.1	23.5	51.40	89.80	46.40	81.40
Variation from controls (%)		+ 56.0		+74.7		+73.9

*Averages of data from 10 experiments on 3 dogs.

TABLE II.
Gastric Secretion Following Double-Histamine Injections.*

Time in min.	Volume (cc)		Total acid (mg HCl)		Free acid (mg HCl)	
	1st Histamine	2nd Histamine	1st Histamine	2nd Histamine	1st Histamine	2nd Histamine
15	1.4	2.0	5.46	5.28	2.91	4.84
30	5.6	5.5	19.49	19.68	17.13	19.56
45	3.5	2.8	12.76	10.52	12.25	9.28
60	1.4	1.2	4.56	4.38	4.49	3.58
75	.9	.6	1.84	1.89	1.68	1.61
90	.7	.4	1.57	1.13	1.30	.88
105	.5	.3	.98	.48	1.32	.30
120	.4	—	.87	—	—	—
Total output	14.40	12.8	47.53	43.40	41.08	40.65
% variation of 2nd from 1st		—11.1		—8.68		—1.04

*Averages of data from 9 experiments on 3 dogs.

output of free hydrochloric acid after the second injection of histamine was 1.04% less than the amount secreted during the test conducted several hours earlier. Only slight differences in total acid and volume were evident. One is, therefore, justified in comparing the output of acid by dogs pre-treated with 929F and injected with histamine with that following a previous injection of histamine the same day. Furthermore, when the average quantity of free or total hydrochloric acid secreted by the dogs after 929F injections was compared with the control output following the second histamine stimulus on other days the increase was approximately 100%.

Thymoxyethyldiethylamine cannot be regarded as a gastric secretory stimulant, for when no histamine was injected the subcutaneous administration of 929F (10 mg per kg) did not stimulate the production of gastric juice. The doses of 929F administered produced no untoward reactions. Larger doses were not employed in any of the experiments dealing with gastric secretion since subcutaneous injections of 20 mg per kg induce retching, vomiting, and defecation in unanesthetized dogs.

Discussion. The ability of 929F to antagonize the stimulating action of histamine on the smooth muscle of bronchioles, uterus, and ileum[3-9] suggested that the drug might inhibit gastric secretion following stimulation of the gastric glands with histamine. Under the conditions of our experiments 929F *failed to inhibit* gastric secretion induced by injecting histamine. Other investigators have used 40 mg of 929F per kg of body weight to protect guinea pigs against lethal doses of histamine (0.5 mg per kg) injected intravenously. Since our animals received a total of only 0.045 mg to 0.07 mg of histamine diphosphate per kg of body weight, subcutaneously, pre-treatment with a smaller dose of 929F should have sufficed to demonstrate the histamine antagonism if it existed in relation to the gastric glands.

The data constitute additional evidence that 929F does not destroy significant amounts of histamine.[5, 6] Furthermore, our results suggest that the mechanism whereby histamine induced contraction of smooth muscle fibers may differ from the mechanism involved in stimulation of gastric glands.

The experimental findings also indicate that 929F, instead of inhibiting gastric secretion induced by histamine administration, *augments* the volume as well as the concentration and total output of hydrochloric acid. This unanticipated augmentation of gastric secretion remains unexplained and is of unknown significance. When no histamine was injected, the subcutaneous administration of 929F failed to induce gastric secretion. The augmenting action of 929F on gastric secretion induced by histamine cannot be due, therefore, to the additive effects of 2 gastric secretory stimulants.

Summary. Studies on gastric secretion in dogs provided with Heidenhain pouches definitely indicated that 929F, given subcutaneously, did not induce gastric secretion. However, following an injection of histamine in animals thus treated, the gastric secretion was markedly increased in volume and acidity over that which was obtained during control periods when only the histamine stimulus was employed.

These findings indicate that 929F, in the doses employed, neither destroys histamine nor antagonizes the action of histamine as a gastric secretory stimulant.

13222 P

Effect of Acetyl Salicylic Acid on Sedimentation Rate of Erythrocytes in Rheumatic Fever.

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Since it is a generally accepted fact that salicyl therapy lowers the temperature and the leucocyte count of patients with acute rheumatic fever, it is our policy to rely on the sedimentation rate of erythrocytes as the best index of disease activity. Only when this returns to normal is the rheumatic infection considered quiescent. Prior to 1940, salicyl therapy was often continued for several weeks after the sedimentation rate became normal, but recently the drug has been discontinued as soon as a normal rate was obtained, assuming that rheumatic activity had then ceased. To our surprise the sedimentation rate in several cases became appreciably elevated during the first week after stopping medication. This was occasionally associated with an elevation of temperature, the return of symptoms, or both. In a few of the cases receiving second or third courses of the drug, the same phenomenon was observed.

The hospital course of 7 patients showing this type of reaction is presented in condensed form in Table I. Each child had acute rheumatic fever, and all except J.S. showed definite cardiac involvement. The rates were determined at approximately weekly intervals

TABLE I.
Effect of Acetyl Salicylic Acid on the Sedimentation Rate.

Patient	J.S.	E.G.	B.P.	R.R.	G.S.	W.M.	G.G.
Age (years), Sex	11, M	6, M	10, F	10, M	14, M	11, M	12, M
Previous attacks	0	0	0	4	2	Several	Several
Dosage (g/day)	2.6	2.6	4.0	3.2	2.6	4	3.2
Duration of treatment (days)	28	14	28	14	9	42	21
Sedimentation rate before and after therapy (mm/min)	1.0-0.2	1.3-0.3	1.8-0.7*	1.1-0.2	0.6-0.2	1.7-0.2	1.5-0.3
Interval between last dose of drug and subsequent determination of rate (days)	4	6	5	6	4	3	3
First sedimentation rate after above interval	1.5	0.6	1.7	0.8	0.5	0.6	0.6
Repetition of phenomenon	2 x	0	2 x	1 x	0	0	0

*Drug discontinued before normal rate was obtained.

using the Rourke-Ernstene[1] technic. This method has been used for several years and has always given apparently consistent and reliable results in the rheumatic patients. Salicyl was administered by mouth in the form of acetyl salicylic acid combined with sodium bicarbonate. The celerity with which the "rise" occurred after discontinuing the drug suggests that this salicyl compound may have the ability to lower the sedimentation rate as well as the temperature and the leucocyte count in this disease.

In seeking an explanation of this reaction, the studies of Bendien, Newberg and Snapper[2] appeared to be pertinent. These authors noted that when sodium salicylate is added to human blood *in vitro,* the erythrocyte sedimentation rate is greatly reduced. In a few preliminary experiments, we have been able to confirm their findings, but the minimal effective concentration of sodium salicylate, using this method, is 90-120 mg %. This represents at least 3 times the value given by Hanzlik[3] as an average blood level obtainable in rheumatic patients.

Further studies are in progress to learn the true mechanism and the significance of this phenomenon.

13223 P

Pigment of Anthracosis.

F. A. TAYLOR. (Introduced by S. R. Haythorn.)

From the William H. Singer Memorial Research Laboratory, Allegheny General Hospital, Pittsburgh.

Black pigment of the human lung, from the time of its earliest observation, was considered to be a collection of coal particles. Pearson in England first applied the term anthracosis in 1813. In Germany, on the contrary, this view was combatted and as late as 1855 Barthelmess discussed the condition as "pulmonary melanosis" and thought it was the result of hemorrhage and inflammation. By 1867 there was general agreement that the pigment was inhaled coal or soot. Klotz[1] has reviewed the subject. No modification of that view appeared until 1940. Then Woodruff[2] and Moerke[3] reverted to the old opinion that the pigment is endogenous. Their pigment was isolated by dissolving the tissue in concentrated hydrochloric acid at room temperature—a procedure known to produce abundant humin in the hydrolysis of protein at the boiling point.

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[1] Rourke, M. D., and Ernstene, A. C., *J. Clin. Invest.*, 1930, **8**, 545.

[2] Bendien, W. M., Newberg, J., and Snapper, I., *Biochem. Z.*, 1932, **247**, 306.

[3] Hanzlik, P. J., *Medicine*, 1926, **5**, 197.

[1] Klotz, O., *Am. J. Pub. Health*, 1914, **4**, 887.

In the present experiments, both clean (calf) and anthracotic lungs (human) were examined for pigment by solution of the tissue in the above solvent as well as in alkaline hypochlorite and by a combination of papain digestion followed by hypochlorite. Only these latter methods sufficed to reduce the clean lung tissue to a negligible residue.

Destruction of uncontaminated lung in concentrated hydrochloric acid left a residue which was soluble in neither organic solvents nor dilute alkali. It was dark red-brown in color, contained 8-10% N in different preparations and 1-1.2% ash. The material of Moerke contained 4-5% N and presumably was a mixture of the above product with anthracotic pigment. As there was no black pigment to be seen in the microscopic examination of the calf lung, the residue is obviously an artefact resulting from the process of digestion of the tissue, and the hydrochloric acid method is not satisfactory for the isolation of the pigment of anthracosis.

When, on the other hand, clean lung tissue was dissolved by treatment with alkaline hypochlorite or papain followed by hypochlorite, and the soaps removed by appropriate solvents, only a trace of insoluble residue was obtained. These procedures, then, constitute satisfactory methods for destroying the tissue. It is not so easy to prove that the pigment will be unaffected. From the character of the pigment finally isolated from black lung, however, it seems extremely unlikely that it would be susceptible to the mild conditions imposed.

In Table I are shown results of analysis of pigments isolated

TABLE I.
Analysis of Pigments.

	Material	N %	Ash %	C %	H %
1	Human Lung	0.4	20.0	65.9	1.4
2	,, ,,		21.6	62.7	1.5
3	,, ,,	0.6	40.0		
4	,, ,,	0.8	49.0		
5	Soot	0.4	15.7		

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[2] Woodruff, C. E., and Moerke, G. A., *Am. J. Path.*, 1940, **16**, Proc., 652.

[3] Moerke, G. A., Abs. of Papers, 99th Meeting Am. Chem. Soc., Cincinnati, 1940, B-2.

from human lungs and for comparison the analysis of a sample of soot from soft coal. In the Kjeldahl digestion for N the pigment can not be dissolved. The figures were obtained by digestion with sulfuric acid until maximum values for N were secured.

It is to be noted that the soot and pigment samples are very similar, the low N figures especially indicating the absence of material derived from tissues, such as was formed in the hydrochloric acid method. In contrast, the residues from clean lungs by the latter procedure contained 8-10% N and only 1-1.2% ash.

If the H of the pigments is calculated as H_2O, then the sum of the C, H_2O and ash obtained by combustion is nearly equal to the quantity taken for analysis. Perhaps if the pigment were dried sufficiently, the H_2O would be decreased.

Although quantitative determinations of pigment in lungs have been reported by several investigators,[1] no previous analysis of the pigment itself has been found in the literature. The present figures leave no doubt that the pigment is exogenous. In both high ash and low N content it resembles soot. If it were melanin, the N content would be in the range of 8-14%.

For the microscopic examination of the tissues, I am indebted to Dr. S. R. Haythorn.

13224 P

A Pharmacological Study of Mannitol Hexanitrate.*

LEON A. HEPPEL. (Introduced by H. C. Hodge.)

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Although mannitol hexanitrate† has been used for many years for the treatment of essential hypertension and as a prophylactic against anginal attacks, few pharmacological studies are available in the literature. This paper is the first of a series designed to test this drug clinically and experimentally.

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* Aided by a grant from the R. J. Strasenburgh Co. The advice of Drs. H. C. Hodge and W. M. Lauter is very much appreciated.

† Due to the chemical instability of mannitol hexanitrate, this compound was used, except where specified, in the form of Maxitate, a stabilized preparation furnished by the R. J. Strasenburgh Co., which contains lactose and starch as diluents.

TABLE I.
Toxicity of Mannitol Hexanitrate on Intraperitoneal Injection into Mice.

No. of mice	Dose, mg	Vol. of solution, cc	No. dead	Mortality, %
24*	5.0	.02	0	0
24*	10.0	.04	2	8
24	12.5	.06	4	17
24	14.6	.07	3	12
16	16.6	.08	4	25
20	18.7	.09	8	40
24	20.8	.10	20	84

For probit kill—5.00, log dose—1.26, or L.D.50—18.2 mg per 20 g mouse. L.D.50 is 0.91 g per kg body weight. (For details of statistical procedure see Bliss.[5])

[5] Bliss, C. L., *Ann. Appl. Biol.*, 1935, **22**, 134.

*Solvent for these 2 groups was mixture of 1 part triacetin and 3 parts acetone. For all other groups the solvent was triethylene glycol. One-tenth cc of triethylene glycol appeared not to disturb a group of 24 mice, while 0.15 cc of this solvent killed 9 out of 12. The triacetin-acetone mixture was more toxic but as much as 0.05 cc was tolerated.

Table I shows that the 24-hour toxicity is very low when tested on 20 g mice. In another experiment, a solution of the material in triethylene glycol was given by stomach tube to 6 male rats weighing about 100 g. None of them developed any symptoms although the dosage was 1.25 g per kilogram.

In a study of chronic toxicity, 12 female rats weighing 60 g were given orally 11 mg per day of mannitol hexanitrate mixed in their diet of Purina fox chow. In addition, 6 male rats weighing 110 g received 8.5 mg in a corn oil solution daily from a medicine dropper. Nine stock animals served as controls. After 24 weeks there was no apparent effect of the drug on the rate of growth, general appearance of the rats or food intake. At autopsy they showed no gross pathological changes. Microscopic sections were made of the following tissues from 12 experimental and 5 control rats: liver, heart, spleen, kidney, and brain. These all appeared normal when stained by the H. and E. method except for moderate splenic congestion. Sections of testis and lung from four experimental rats showed no abnormalities.

Clinically it has been observed that the depressant effect of mannitol hexanitrate is more prolonged than that of other nitrate esters, *e. g.*, glyceryl trinitrate and erythrol tetranitrate.[1] Yet the action of these compounds when injected intravenously into anesthetized cats was shown to be evanescent and similar in the blood pressure lowering, confirming the work of Herrman, *et al.*[2] The prolonged action of mannitol hexanitrate may depend on slowness of absorption. To test this point the following experiments were carried out with glyceryl trinitrate and mannitol hexanitrate: Young female rats were starved for 48 hours and then given from 15 to 20 mg of the drug in triethylene glycol solution by stomach tube. After varying intervals of time the rats were killed and the entire gut was carefully dissected out, following the Cori technic.[3] Extraction was then carried out with 100 cc ether, after which an aliquot was evaporated to dryness and hydrolyzed with warm 1N sodium hydroxide solution. The Stieglitz and Palmer method[4] was then used with slight modifications to analyze for nitrates.

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[1] Lawrence, G. H., *Arch. Int. Med.*, 1912, **9**, 409.

[2] Herrman, R. F., Leake, C. D., Loevenhart, A. S., and Muehlberger, C. W., *Proc. Am. Soc. Pharm. and Exp. Therap.*, 1926, **27**, 259.

Glyceryl trinitrate was completely removed from the gastrointestinal tract within 6 hours in the case of 6 rats out of 7. In one instance removal was 60% completed. Four rats showed from 40 to 90% absorption during a 3-hour period. In the case of mannitol hexanitrate, 3 rats showed 20-30% absorption at the end of 3 hours and 6 rats showed 0-20% absorption after 6 hours. Apparently glyceryl trinitrate is taken up much more rapidly than mannitol hexanitrate.

13225

Influence on Development of Certain Dominant Lethals Induced by X-rays in Drosophila Germ Cells.

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The action of radiant energy in inducing gene mutations and various types of chromosomal alterations has been known for several years.[1, 2] The alterations produced have involved changes in parts of chromosomes, whole chromosomes and apparently in the composition of individual genes. In Muller's classic report[1] on the artificial production by X-rays of alterations in the hereditary material of *Drosophila melanogaster,* evidence was presented, through breeding experiments, of the occurrence of dominant lethals. These are lethals whose presence in single dose can cause the death at various stages of development of the zygotes which contain them and thus are never transmitted to succeeding generations. Such dominant lethal genetic changes may be induced by X-rays in germ cells and then contributed to the zygote by one of the gametic nuclei. As yet, however, little is known of how the inviable nuclear alterations act to modify development. It is the purpose of this report to deal with certain aspects of this question.

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[3] Cori, C. F., *J. Biol. Chem.*, 1935, **66**, 691.

[4] Stieglitz, E. J., and Palmer, A. E., *J. Pharm. and Exp. Therap.*, 1934, **51**, 398.

* Fellow, John Simon Guggenheim Memorial Foundation.

† Senior Radiobiologist, National Cancer Institute, Bethesda, Maryland.

[1] Muller, H. J., *Science*, 1927, **66**, 84.

[2] Duggar, B. M., Editor, *Biological Effects of Radiation*, Vol. 2, Chaps. 38 and 39, McGraw-Hill, New York, 1936.

The random effects of the radiations on the chromosomes of Drosophila have been reported elsewhere.[3] It was observed that the meiotic and mitotic divisions can become drastically disordered following treatment of the germ cells with X-rays. The achromatic figures as well as the chromosomes proper are frequently aberrant. In the zygotic mitoses chromosome fragmentation and elimination, asymmetrical divisions, multipolar divisions of the tripolar and quadripolar types and pycnotic, clumped chromosomal complexes with occasional chromatic bridges were noted and described. These induced abnormalities were considered to be directly responsible for the greatly increased percentage of mortality among the offspring, since the frequency of organisms showing such mitotic abnormalities was found to be about equal to the frequency of zygotic lethals.

For the present work, groups of wild type adult *Drosophila melanogaster* of both sexes, in separate gelatin capsules, were given from 2340 to 4680 r of hard (200 K.V.) X-rays. Subsequently, the treated flies were mated with untreated ones and eggs were collected and fixed at timed periods of development. Thus, each organism commenced development with one haploid chromosomal complement contributed by an irradiated parent and the other by a non-irradiated mate. All cells, germinal and somatic, are descended from the original fusion nucleus containing both the treated and untreated sets of chromosomes. Figs. 1-3 represent sections of 6 organisms which were of comparable age, all having incubated from 12 to 16 hours. Sections of 2 normal embryos are shown in Fig. 1, of 2 embryos whose mothers had been treated in Fig. 2, and of 2 embryos whose fathers had been treated in Fig. 3.

In the non-irradiated controls of our experiment (Fig. 1), the alimentary tract is differentiated into mouth, pharynx, esophagus,

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[3] Sonnenblick, B. P., *Proc. Nat. Acad. Sci.,* 1940, **26**, 373.

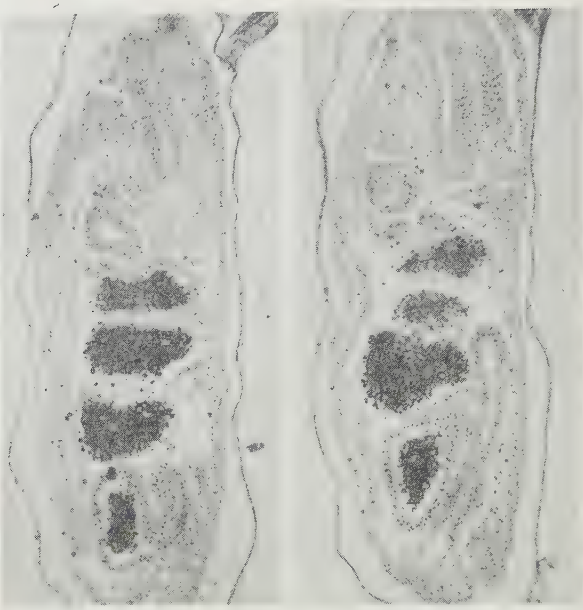

FIG. 1.

Sections of 2 normal embryos. Differentiation far advanced; hypodermis present, nervous tissue becoming concentrated, fore-gut and hind-gut wall well developed, deeply staining yolk spheres enclosed in loops of mid-gut.

proventriculus and gut, loops of which are shown in the sections to enclose the deeply staining yolk spheres. Nervous tissue is becoming more concentrated and nerve fibers are present. The hypodermis is delimited and the frontal sacs from which the optic discs are to be derived have made their appearance. These relatively complex normal organisms differ strikingly from those which contain the treated chromosomes (Figs. 2, 3). In the latter there is no gut and the yolk is free, concentrated in one or another part of the embryo and unincorporated in the mid-gut. Part of the cytoplasm may remain non-cellular, taking a deep stain (Fig. 2). Cell formation, which occurs normally over the entire surface of the early egg, has obviously been interfered with. In none of these organisms were germ cells, the first differentiations evident in the developing egg, to be noted. Masses of small cells, with occasional large spherical cells interspersed among them, make up the remainder of the embryos. Inasmuch as no differentiation or structural organization is to be observed, one may safely conclude that such animals will not hatch from the embryonic envelopes as active larvae. It is an extraordinary fact that the powers of differentiation can be so completely destroyed without at the same time destroying the powers

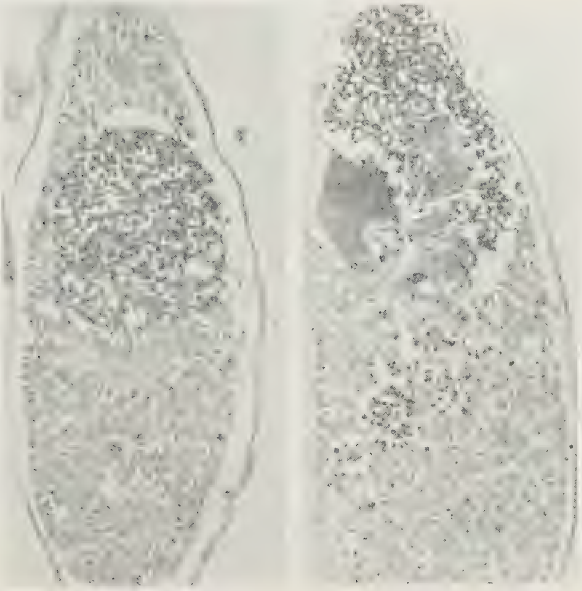

FIG. 2.

Sections of embryos derived from treated mothers and untreated fathers. No structural organization present although of same age as embryos shown in first figure. Except for the unenclosed yolk masses, the organism is virtually a sac of cells. A portion of the cytoplasm may remain non-cellular.

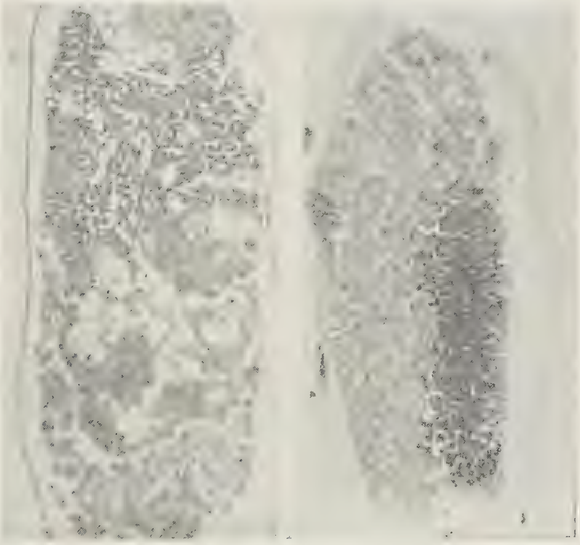

FIG. 3.

Sections of embryos derived from treated fathers and untreated mothers. No tissues or organs are evident. Unenclosed yolk inclusions, regions of non-cellular cytoplasm and huge vacuoles are present.

of cellular proliferation. It should be stated, however, that while the illustrated instances of unregulated proliferation are extreme cases, such are by no means rare. In 182 embryos which were sectioned and studied, we observed this effect 6 times, indicating that 1 out of approximately 30 treated germ cells had been so altered by the X-rays that subsequent differentiation went completely awry while the cells derived from the treated gamete could still multiply for many cell-generations. A somewhat similar result has been obtained by irradiating diploid cleavage nuclei.[4] However, in that instance a variable number of nuclei were exposed and the ensuing events could not be analyzed in terms of the effect on a single nucleus as in the observations reported here.

Technics exist whereby the Drosophila geneticist can breed strains in which identified chromosomes or sections of chromosomes are present as duplications, or are translocated, or are even absent entirely. Effects on development of certain genes or blocks of genes may thus be determined. Poulson[5] has recently studied the development of Drosophila eggs which lack the entire X-chromosome. There is an irregular disposition of the syncytial cleavage nuclei and no blastoderm is formed. Cell multiplication proceeds, however, and in sections of eggs which have incubated for more than 10 hours, a stratification of materials is observed with cellular aggregates, non-cellular cytoplasm and unincorporated yolk in the different regions. The absence of a known chromosome, or, to put it another way, the lack of the gene complex of that chromosome has resulted in an extensive but unregulated cellular proliferation and eventual death of the zygote. With this study of Poulson's in mind and recalling the effects of X-rays on Drosophila chromosomes mentioned above, it would appear that the loss of a relatively large block of genes through chromosome breakage and elimination, or through the abnormal distribution of chromosomes and chromosome fragments, has been a primary factor in the initiation of the processes leading to uncontrolled cellular divisions and failure to differentiate.

The problem is hardly confined to Drosophila for the induction with X-rays of dominant lethal nuclear alterations has been reported in other animals and in plants. Conclusive evidence for their production in mammals was obtained by Snell.[6, 7] The reduction in productivity in litters sired by irradiated male mice he attributed to lethal genetic alterations in the exposed spermatozoa. Subsequent analysis by Snell of several generations of offspring indicated a high incidence in the sperm of chromosomal aberrations of the type known as translocations. Dominant lethal alterations resulting in abortion of the embryos have also been induced in maize pollen by X-rays and ultraviolet radiation.[8] No cytologic or embryologic examination of such abortive embryos has as yet been reported.

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[4] Henshaw, P. S., *Radiology,* 1935, **24**, 438.

[5] Poulson, D. F., *J. Exp. Zool.,* 1940, **83**, 271.

[6] Snell, G. D., *Proc. 6th Int. Congress of Genetics,* 1932, **2**, 188.

[7] Snell, G. D., *Am. Nat.,* 1933, **67**, 81.

In conclusion, therefore, it should be emphasized that it is possible to demonstrate one type of dominant lethal effect whereby cells are not impaired in their ability to proliferate while their ability to differentiate is at the same time completely destroyed. The lethal effect, it would appear, is probably due to the absence or deficiency of parts of chromosomes, and therefore of gene loci, necessary for the development of the organism. Important implications arise from these considerations since somatic as well as germ cells may be affected by radiation in a manner similar to that discussed in this paper with resulting proliferative growth unaccompanied by any differentiation.

13226

Specificity of Fowl and Mammalian Antigonadotropic Sera.*

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(Introduced by R. K. Meyer.)

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Numerous investigators have found that the injections of gonadotropic extracts over long periods of time result in the production of a substance in the sera of the treated animals that neutralizes the gonad-stimulating action of the injected extract. The antisera obtained from animals repeatedly injected with gonadotropic preparations from either beef or sheep pituitary glands inhibit not only the extract used in the production of the antisera but also various other gonadotropic extracts, such as human, rat and horse pituitary;

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[8] Stadler, L. J., *Proc. 7th Int. Congress of Genetics*, 1939, 269.

* Supported in part by a grant-in-aid from the Wisconsin Alumni Research Foundation administered by Roland K. Meyer. Assistance was also furnished by the personnel of the W.P.A. Official Project No. 65-1-53-2349.

† Present address: Lakeside Laboratories, Milwaukee, Wisconsin.

human pregnancy urine and pregnant mare serum. In addition the aspecific antisera are capable of suppressing the endogenous gonadotropic secretion of the rat, rabbit and dog pituitary. A discussion of these data will be found in recent review papers on antihormones.[1, 2] A tentative explanation has been offered by Parkes and Rowlands[3] to explain the phenomena whereby an antisera for the pituitary gonadotropic hormone from one animal is capable of inhibiting both exogenous gonad-stimulating preparations and endogenous gonadotropic secretion of the pituitary gland from other animals. Their hypothesis states that the gonadotropic complex of extracts when injected into rabbits acts as a haptene and its association with heterologous protein makes it capable of producing aspecific antigonadotropic substances. Parkes and Rowlands[3] found, however, that beef antigonadotropic serum although effectively inhibiting gonadotropic secretions and extracts from mammalian pituitaries, serum or urine, is without action against the endogenous gonadotropic secretion of the chicken hypophysis.

In an attempt to obtain more information concerning the specificity of antigonadotropic substances experiments were designed to determine whether antisera developed against the gonad-stimulating hormone of chicken or sheep pituitaries would prevent the gonadotropic effect of mammalian gonadotropins and extracts of chicken pituitary glands.

Procedure. The sheep antigonadotropic sera were obtained from rabbits repeatedly injected with an aqueous, supercentrifuged extract of sheep pituitary glands. The rabbits were injected with 250 mg equivalent of dried powder per day for the first 25 days, after which the same dose was administered every second day for 3 months or longer. The chicken antigonadotropic serum was prepared by injecting an aqueous suspension of 25 mg of air-dried chicken pituitary powder into rabbits every day for the first 25 days, followed by injections of the same dose every second day for 4 months. The blood was obtained by cardiac puncture and the serum prepared by centrifugation.

The inhibitory qualities of the antisera were determined by the ability of the sera to prevent the action of the gonad-stimulating extracts in rats when the serum and the extract were simultaneously administered by separate injections to 21-day-old female rats in 9 equal doses of 0.5 cc during a period of 4.5 days. Autopsy was

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[1] Collip, J. B., Selye, H., and Thomson, D. L., *Biol. Rev.*, 1940, **15**, 1.

[2] Thomson, D. L., Collip, J. B., and Selye, H., *J. A. M. A.*, 1941, **116**, 132.

[3] Parkes, A. S., and Rowlands, I. W., *J. Physiol.*, 1937, **90**, 100.

done during the morning of the sixth day and the ovaries of the rats were weighed to the nearest milligram. The aspecificity of the sheep antigonadotropic serum was determined by its inhibitory effect on various gonadotropic extracts other than that made from sheep pituitaries.

Two lots (A and B) of sheep antigonadotropic rabbit serum were used. "A" represents the serum from a single rabbit while "B" is the pooled serum from several rabbits repeatedly injected with sheep pituitary extract. Serum A was injected into rats with the following gonadotropic preparations: aqueous extracts of fresh and acetone-dried sheep pituitaries, suspensions of dried human pituitary glands, suspensions of chicken pituitary glands, pregnant mare serum and an extract of human pregnancy urine made by alcohol precipitation. Serum B was injected with sheep, rat and chicken pituitary extracts and purified pregnant mare serum[4] and purified human pregnancy urine extract obtained by benzoic acid adsorption.

The chicken antigonadotropic rabbit serum was obtained from one rabbit only and was tested for antigonadotropic action by injecting it with sheep and chicken pituitary gland extracts.

Results and Discussion. The data concerning the inhibitory action of antiserum A and B and antichicken rabbit serum are recorded in Table I. The sheep antigonadotropic rabbit sera were effective in completely neutralizing the gonadotropic activity of the extracts from mammalian sources but were ineffective against the gonad-stimulating action of chicken pituitary glands. The serum of the rabbit repeatedly injected with the suspension of chicken pituitary powder effectively inhibited the action of the gonadotropic hormone of chicken hypophyses, but no demonstrable inhibitory effect could be detected when the chicken antigonadotropic rabbit serum was injected with sheep pituitary extracts.

The gonadotropic effects of the chicken and mammalian preparations are essentially the same when administered to 21-day-old female rats since each produces follicles and corpora lutea.[5, 6] However, when an antihormone is formed against these extracts, although both produce ovarian growth in mammals, a definite class specificity is noted as regards the neutralizing action of the sera. This relationship of class specificity and order aspecificity of

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[4] Cartland, G. F., and Nelson, J. W., *J. Biol. Chem.*, 1937, **119**, 59.

[5] Meyer, R. K., Mellish, C. H., and Kupperman, H. S., *J. Pharm. and Exp. Therap.*, 1939, **65**, 104.

[6] Leonard, S. L., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 566.

TABLE I.
Effect of Chicken and Sheep Antihormone on Gonad-stimulating Action of Mammalian and Chicken Gonadotropins.

Gonadotropic substance	Amt	Type of antisera	Dose of antisera, cc	Avg ovarian wt	
				Gonadotropic substance	Gonadotropic substance + antisera
Sheep pituit. ext.	100 mg equivalent of powder	AntiSAP* A	2	69 (6)‡	10.5 (4)‡
Sheep pituit.—fresh susp. of whole gland	200 mg	” A	2	38 (3)	9.0 (2)
Human pituitary	5 mg air-dried powder	” A	2	42.5 (9)	17.5 (2)
Human pregnancy urine ext.	35 mg powder	” A	2	40 (4)	16.5 (4)
Pregnant mare serum	0.25 cc	” A	2	38 (3)	9.0 (2)
Chicken pit.—fresh susp. of whole gland	200 mg	” A	2	48 (8)	37.5 (8)
Sheep pituit. ext.	100 mg equivalent of powder	” B	1	74 (11)	14 (8)
Purified human pregnancy urine ext.	1 mg powder	” B	2	44 (4)	15 (4)
Purified pregnant mare serum	0.5 mg powder	” B	2	184 (4)	8 (4)
Rat pituitary	2 mg air-dried powder	” B	2	58 (4)	9 (4)
Chicken pituit.	40 mg ” ” ”	” B	2	53 (9)	85.6 (10)
” ”	40 mg ” ” ”	AntiCAP†	2	53 (9)	14 (6)
Sheep pituit. ext.	100 mg equivalent of powder	”	2	85 (4)	102 (5)

*Sheep antigonadotropic rabbit serum.
†Chicken antigonadotropic rabbit serum.
‡Figure in parenthesis indicates the number of rats used.

mammalian and aves antigonadotropic sera is like that found in serological relationships where interclass specificity and interorder aspecificity are noted in the study of classical antigen-antibody reactions. The results reported herein suggest that the gonadotropic complex of chicken hypophysis is immunologically distinct from that observed for the mammalian gland and give further support to the theory that antigonadotropic substances are immune bodies.

Summary. Sheep antigonadotropic rabbit serum, aspecific in nature, although effective in inhibiting the gonadotropic action of extracts of mammalian tissues and fluids did not prevent the typical effect of gonadotropic extracts made from chicken hypophyses. Chicken antigonadotropic rabbit serum, while inhibiting the gonadotropic action of chicken pituitary glands, was ineffective against gonadotropic extracts of sheep pituitary glands. The possible relationship of these experiments are discussed in regard to the nature of the antihormone mechanism.

13227

Effects of Estrone and Progesterone on Male Rabbit Mammary Glands. I. Varying Doses of Progesterone.

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From the Division of Anatomy, University of California Medical School, Berkeley, and the Research and Biological Laboratories, Parke, Davis, and Company, Detroit, Mich.

Turner and Frank[1] showed that whereas estrogen caused growth of the duct system and slight lobule proliferation in the male rabbit mammary gland, the combination of estrogen and progestogen caused lobule-alveolar growth simulating that seen in pregnancy. The hormones used were impure, but were assayed for rat units of estrogen and rabbit units of progestogen and judged by the results, were uncontaminated, one with the other. Until recently few investigators have had at their disposal sufficient progesterone with which to investigate this problem further, and, as far as we are aware, the doses of estrone and progesterone that will synergize best to cause optimal mammary growth have not as yet been satisfactorily determined in any animal form. In the preliminary investigation reported herein, an attempt was made to determine the approximate dose of progesterone that would function optimally with a given daily dose (120 I.U.) of estrone in causing prolactational* growth of the male rabbit mammary gland.

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[1] Turner, C. W., and Frank, A. H., *Mo. Agr. Exp. Res. Bull.* 174, 1932.

Experimental. Immature, New Zealand White male rabbits weighing approximately 1.5 kg at the beginning of the experiment were treated in groups of 3 with 18 daily doses (Monday through Friday, from 2/28/39 until 3/23/39) of the following:

Group 1, 120 I.U. of estrone (theelin); Group 2, 1 I.U. of progesterone; Group 3, 120 I.U. of E and 0.25 I.U. of P; Group 4, 120 I. U. of E and 1 I.U. of P; Group 5, 120 I.U. of E and 4 I.U. of P; Group 6, 120 I.U. of E and 8 I.U. of P. The 2 hormones were given separately, subcutaneously, in peanut oil. On 3/28/39, 5 days after the last injection, a biopsy specimen of the second left (thoracic) mammary gland was taken from each animal, fixed in formol, stained *in toto* with alum-carmine and cleared in methyl salicylate.

Results. The mammary spreads from Group 1, showed that the dose of 120 I.U. of E produced good duct growth with almost negligible alveolar formation (Fig. 2). Those from Group 2 (1 I.U. of P) showed but little more development than that seen in normal immature rabbit glands (Fig. 1). The duct system of the glands from animals in Group 3 (120 I.U. of E plus 0.25 I.U. of P) was as extensive as that seen in animals treated with 120 I.U. of E alone, but the main ducts were narrower and, as though in compensation, more alveolar buds were present. Thus, just as progesterone in some experimental animals prevents the estrogen-induced uterine dilatation and stimulates a proliferation of luminal and glandular epithelium, so also in the rabbit it counteracts an estrogen-induced mammary duct dilatation and permits instead an extensive alveolar proliferation. The amount of incomplete lobule formation shown in Fig. 3 was found typical of all rabbits in this group and probably may be interpreted as barely minimal prolactational proliferation in response to a low dose of progesterone. The glands from animals in Group 4 (120 I.U. of E and 1.0 I.U. of P) showed the best evidence of synergism obtained in this preliminary experiment (Fig. 4), although judged on the basis of a set of glands taken at different stages of pregnancy, they could not be said to show maximal prolactational proliferation. Interesting results were obtained in Groups 5 and 6, where 2 of the animals in Group 5 and all in Group 6 showed only scanty alveolar development and an inhibition of the duct growth (Fig. 5). The third animal in Group 5 showed good prolactational development with no inhibition of duct growth, indicating that 4 I.U. of progesterone approximated the border-line inhibiting dose when given with 120 I.U. of estrone by this particular routine.

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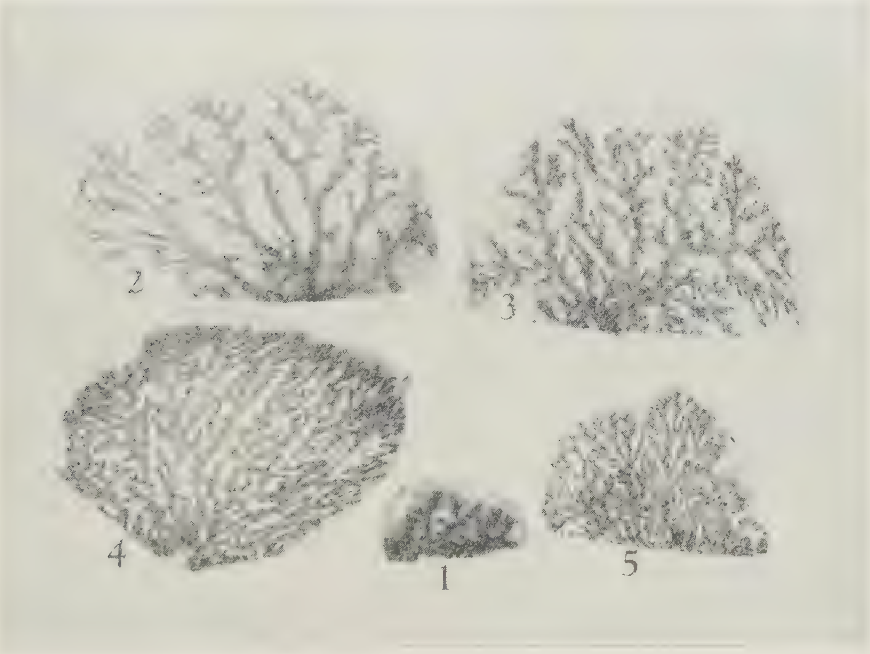
* The term prolactational is used to indicate the additive effect of progesterone on the mammary gland (lobule-alveolar growth) in the same sense that progestational is used for the progesterone effect on the rabbit uterus. In the rabbit usually by the third week of pregnancy, maximal prolactational growth is attained through a rapid multiplication of alveoli in large complex lobules. The term lactational growth is used for the enlargement of individual alveoli that takes place at parturition (sometimes before) under the direct influence of hypophyseal lactogenic hormone.

Summary. Eighteen doses of 0.25, 1.0, 4.0 and 8.0 I.U. of crystalline progesterone were given, simultaneously with 120 I.U. of



All figures represent approximately one-half of a male rabbit mammary spread fixed in formol and stained in alum-carmine. × 1.5.

Treatment was as follows:

FIG. 1. None. Control gland.

FIG. 2. 18 subcutaneous doses of 120 I.U. estrone in oil over a 28-day period. Duct growth with almost no alveolar development.

FIG. 3. 18 subcutaneous doses of 120 I.U. estrone and 0.25 I.U. progesterone over same period. Very little prolactational proliferation.

FIG. 4. 18 subcutaneous doses of 120 I.U. estrone and 1 I.U. progesterone over same period. Fair prolactational proliferation.

FIG. 5. 18 subcutaneous doses of 120 I.U. estrone and 8 I.U. progesterone over same period. Inhibited duct growth and only slight alveolar development.

estrone, to immature male rabbits during a period of 4 weeks. Of these 4 levels of progesterone, the 1.0 I.U. dose synergized best although the prolactational proliferation induced was not maximal. The 4.0 and 8.0 I.U. doses of progesterone were apparently inhibiting as shown by the relatively poor mammary growth obtained.

13228

Effects of Estrone and Progesterone on Male Rabbit Mammary Glands. II. Varying Doses of Estrone.*

George Scharf and Wm. R. Lyons.

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In the preceding paper it was shown that of 4 different levels of progesterone, only one (1 I.U.) functioned well with a given daily dosage of estrone (120 I.U.) in producing prolactational growth of male rabbit mammary glands. A lower dose was insufficient and higher levels were inhibitory. The inhibition of estrin effects by progestin has received considerable attention as far as uterine and vaginal reactions have been concerned, but almost no work of a similar nature has been done on the mammary gland. It is also well known that estrogen may inhibit the progestin effect on the uterus, but again almost no information is available regarding estrogen inhibition of the progestin effect on the mammary apparatus.

Experimental. Continuing our attempt to determine the optimal combination of estrone and progesterone for prolactational proliferation of male rabbit mammary glands, the daily dose of progesterone found best in the preceding experiment (1 I.U. subcutaneously in sesame oil) was held constant while estrone was given simultaneously, though separately at the following levels to 6 groups of 3 rabbits each: 30, 60, 120, 240, 480, and 960 I.U. daily subcutaneously in sesame oil, 5 days weekly (Monday through Friday) for 5 weeks. These 6 levels of estrone alone were injected likewise into 6 groups of 3 rabbits each. On the day following the last injection, mammary glands were taken at biopsy or necropsy and

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* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

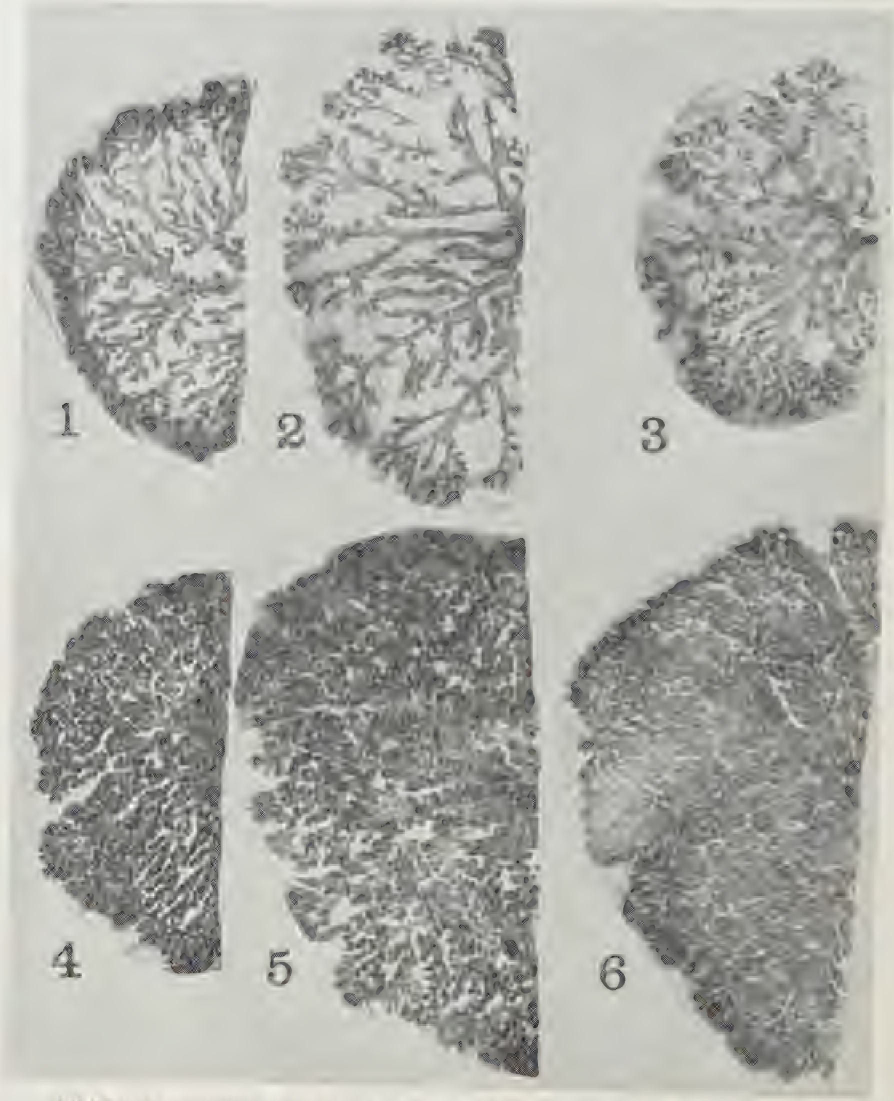
The estrone and progesterone were generously supplied by Parke, Davis and Company, Detroit, Michigan.

handled as previously described. For comparative purposes, a series of mammary spreads were obtained from females of the same strain of New Zealand White rabbits at weekly intervals during their first pregnancy.

Results. The mammary reactions to E or E plus P were quite constant within groups, care having always been taken to compare glands taken from the same region. The 30 and 60 I.U. daily doses of E induced a mammary growth that was somewhat less extensive (Fig. 1) than that obtained with the 120, 240, and 480 I.U. levels which were all about equally effective (Fig. 2) with an optimum at 120 I.U. The 960 I.U. level was excessive as indicated by relatively less duct extension as well as by cyst-formation observed in this group (Fig. 3). This tendency to cyst-formation or sacculation in the larger ducts was evident even in the 240 I.U. level as may be seen in Fig. 2.

When 1 I.U. of P was given simultaneously with the different levels of E, prolactational proliferation was observed in every case, although considerable variation in the number and completeness of the lobules was encountered. In the groups receiving 1 I.U. of P plus 30 and 60 I.U. of E respectively, good lobule-formation was observed (Fig. 4), but the duct system had not extended itself as much as in the rabbits receiving the higher levels of E (Estrone) along with P (Progesterone). This finding was consistent with the results obtained with 30 and 60 I.U. of E alone, where inferior duct growth was produced. In the groups receiving the 4 higher levels of E plus P, duct growth was extensive and apparently maximal for the routine used since all 4 levels produced duct systems approximating each other in length. However, it was observed that the groups receiving 1 I.U. of P plus 240 and 960 I.U. of E respectively showed better lobule formation than those receiving 1 I.U. of P plus 120 and 480 I.U. of E respectively. In the latter 2 groups there were more incomplete lobules in which alveoli seemed to be still in process of multiplication and the final "grape-cluster" arrangement had not yet been attained. In glands from animals receiving 240 I.U. of E plus 1 I.U. of P complete lobules predominated but some incomplete lobules were also present, as may be seen in the lower quarter of the gland shown in Fig. 5. The glands from rabbits receiving 960 I.U. of E plus 1 I.U. of P showed the greatest lobular proliferation (Fig. 6), and in striking contrast to the animals receiving 960 I.U. of estrone alone showed neither cyst formation nor inhibition of duct extension.

In none of the experimental groups did the results parallel pre-



All figures represent approximately one half of a male rabbit mammary spread fixed in formol and stained in alum-carmine. × 1.5.

Daily treatment with estrone and progesterone injected subcutaneously in oil 5 days each week for 5 weeks was as follows:

FIG. 1. 30 I.U. of E.
FIG. 2. 240 I.U. of E.
FIG. 3. 960 I.U. of E.
FIG. 4. 30 I.U. of E plus 1 I.U. of P.
FIG. 5. 240 I.U. of E plus 1 I.U. of P.
FIG. 6. 960 I.U. of E plus 1 I.U. of P.

cisely the complete prolactational proliferation attained between the second and third weeks of pregnancy, but the groups receiving 240 I.U. of E plus 1 I.U. of P and 960 I.U. of E plus 1 I.U. of P most closely approximated that type of growth. The main difference in the gland shown in Fig. 6 and that of a 2-3-week pregnancy gland is revealed at the periphery where lobule formation in the experimental animal is incomplete and growing club-endings are prominent. At the third week of pregnancy, adjacent mammary glands usually have overlapped at their peripheries and club-endings have been transformed into complete lobules. However, it should be remembered that at the beginning of pregnancy an extensive duct system already exists whereas at the beginning of the injection period in our male rabbits an extremely rudimentary gland was present (Fig. 1 of preceding paper). Another difference had to do with the overlapping of lobules within a gland—a process which gives rise in part to the increase in thickness of the gland in the latter half of pregnancy. In Fig. 6 and to a lesser degree in Fig. 5 this overlapping may be seen, but again it was not quite as extensive as in the glands of 3-week pregnant rabbits.

Summary. Six groups of immature male rabbits were injected 5 days weekly for 5 weeks with 30, 60, 120, 240, 480, and 960 I.U. of estrone respectively. The best mammary duct growth occurred in the animals receiving 120 I.U. The 30 and 60 I.U. animals showed slightly less extensive duct systems, and the higher levels caused cystic changes in the main ducts.

The same 6 levels of estrone were injected into 6 other groups of male rabbits along with 1 I.U. of progesterone daily for a similar period. In every case, lobular growth of the mammary glands occurred and no tendency toward cyst formation was noticed even in the animals receiving the highest level of estrone. The mammary glands of the animals receiving the 2 lowest levels of estrone, plus progesterone had not developed as extensively as the others, but showed good lobule formation on a relatively shorter duct system. The best prolactational proliferation was observed in the groups receiving 1 I.U. of progesterone plus 240 and 960 I.U. of estrone respectively. Only the presence of incomplete lobules at the periphery of these glands made possible their differentiation from glands taken from rabbits in their second to third week of pregnancy—a period when prolactational proliferation is usually maximal.

13229

Cultivation of *Trypanosoma equiperdum* in Yolk-Sac of Developing Chick Embryo.

E. Alture-Werber. (Introduced by Geoffrey Rake.)

From the Division of Microbiology, The Squibb Institute for Medical Research, New Brunswick, N. J.

Chabaud[1] cultivated *Trypanosoma rhodesiense* on the chorio-allantoic membrane of 7- to 10-day-old chicken embryos using citrated or defibrinated mouse blood containing about 50 trypanosomes per field. All of the eggs inoculated with the citrated blood became infected, but only 50% of those inoculated with the defibrinated blood. Trypanosomes could be found 96 hours after inoculation and the number of them increased until the 6th day, whereafter they diminished, and finally disappeared by the 9th day. The average life time of the embryo was 7 days, but some survived for 9, 10, and 11 days. Chabaud also was successful in cultivating *T. brucei* and *T. equinum* in the egg. With both, the embryos first showed infection in 86 to 90 hours and lived an average of 7 to 9 days. With *T. lewisi* he could not obtain any infection of the chick embryo although he incubated the infected eggs for 5 to 15 days. Longley, Clausen, and Tatum[2] inoculated *T. rhodesiense* into the allantoic cavity of 8- to 10-day-old chicken embryos. The embryos usually died 5 days after inoculation with a heavy infection in the blood. These authors were able to transfer this strain through 8 subcultures by using blood diluted with 0.9% saline during 41 days. They were also able to cultivate *T. equiperdum, T. brucei, T. evansi*, and *T. hippicum* in the same way. These trial experiments were carried on for 15 days. Oag[3] inoculated series of chick embryos, in age from 8 to 12 days, with *T. brucei, T. congolense* and *T. equiperdum*. Neither with *T. congolense* nor *T. equiperdum* could the protozoa be demonstrated after incubation. Seven eggs infected with *T. brucei* showed living trypanosomes 3 to 4 days after incubation. Blood of these positive eggs passed to another set of eggs gave no results. He assumed that his results were due to survival of the protozoa during the 3 to 4 days' incubation. No details of method are given.

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[1] Chabaud, A., *Bull. Soc. path. exot.*, 1939, **32**, 489.

[2] Longley, B. J., Clausen, N. M., and Tatum, A. L., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 365.

[3] Oag, R. Knight, *J. Path. and Bact.*, 1940, **51**, 137.

In connection with studies on the effect of arsenical compounds in syphilis and trypanosomal infections the possibilities of cultivating *T. equiperdum* in the egg were investigated. A series of experiments was made using 6- to 10-day-old chick embryos. The infection was performed by injection of citrated mouse blood containing *T. equiperdum* diluted with infusion broth into the yolk sac of the egg. In the first experiment a dose of 0.5 cc diluted mouse blood, containing 1 to 2 trypanosomes per microscopic field (magnification 98×7.5) was used. In the following experiments a counted number of trypanosomes were inoculated. It was found that *T. equiperdum* inoculated in the egg could only be demonstrated subsequently if inocula containing 500,000 or more protozoa were used. Of the different dosages used 2 million trypanosomes per egg in ½ cc broth (or 4 per field) gave the most satisfactory results.

When 2 million *T. equiperdum* per egg were used, 14 out of 30 eggs died between the 7th and 11th day. Between the first and 10th day after infection 16 eggs were opened, of which 2 were negative both microscopically and by mouse test. The infected eggs opened 1, 2, and 3 days after inoculation did not reveal any trypanosomes microscopically in the embryo blood, or blood of the chorio-allantoic membrane vessels. (The mixture of allantoic fluid and blood is referred to as fluid in this paper.) Most mice injected with either the yolk or fluid of these early opened eggs became infected and died between 7 and 10 days. Among 4 eggs opened 4 and 5 days after inoculation 2 eggs were negative microscopically both in the yolk and in the fluid, while the other 2 showed trypanosomes in the fluid but none in the yolk. The 2 negative fluid specimens killed mice in 5 days and the 4 yolk specimens killed mice in 7 to 13 days with infection due to *T. equiperdum.* Eggs opened 7 days after inoculation had 12 to 21 parasites per field in their blood. The yolk of these eggs killed mice in 7 to 14 days. Four eggs opened 8 and 9 days after infection showed between 5 and 25 parasites per field in the fluid. Mice inoculated with the yolk of the last mentioned eggs died in 5 to 7 days. Occasionally trypanosomes were found in wet and stained yolk preparations of infected eggs. In all the experiments the consistency of the yolk made the microscopic demonstration of trypanosomes very difficult. Death of the chick embryo was usually associated with hemorrhages in the brain, liver, and pericardium. Once a large hemorrhage occurred on the chorio-allantoic membrane. Twice hemorrhages were found in living embryos. If embryos were examined shortly after death trypanosomes could be found by microscopic examination. However, a few hours later the infection could be traced only by mouse passage and if too much time passed before inoculation in mice, no infection in mice could be obtained. Sometimes it took 15, 18, 29, and once 35 days to kill mice with *T. equiperdum* infection.

An inoculum of one million trypanosomes per egg also caused infection in the embryo. High numbers of trypanosomes were found in the blood of the embryo and in the fluid. The embryos died between the 8th and 11th day after infection. Twelve eggs infected with 500,000 trypanosomes gave the following results: nine embryos died between the 9th and 11th day with hemorrhages in the brain and in the pericardium, while 2 eggs opened on the 9th and 11th day did not show any infection microscopically. Unfortunately no mouse passages were performed in this experiment. One egg opened on the 12th day had numerous trypanosomes in its blood and fluid. The fluid count was 198 million per cc. Eggs infected with 150,000 trypanosomes and less showed no infection either microscopically or in mouse passage, although a great number of eggs died during the experiments.

Summary. Trypanosoma equiperdum can be cultivated in the developing chick egg by inoculation of the infected material into the yolk sac of the egg. An inoculum of 2 million trypanosomes per egg gave satisfactory results. In 93% of infected eggs microscopically visible infection occurred, or infection could be demonstrated by mouse passage. When trypanosomes could not be found microscopically in the egg, mice inoculated with the egg specimen usually became infected. Sometimes it took 14 to 35 days to have such mice die with *T. equiperdum* infection. Trypanosomes which did not infect the embryos seemed to have been destroyed or retained in the yolk sac. Death of infected embryos was accompanied with hemorrhages in the brain, liver, and heart. Inocula of 500,000 and one million trypanosomes caused infection in the egg. With inocula of 150,000 or less organisms satisfactory results were not obtained. Repeated passages in the egg do not change the virulence of *T. equiperdum*. To maintain strains in eggs it is necessary to inoculate a sufficient dose, *i. e.,* 2 million organisms, to use several eggs, and to transfer once a week. The advantages of the above method are: 1. The injection into the yolk sac is a simpler method than injection on the chorio-allantoic membrane. 2. By injection into the allantoic cavity the embryo is killed in (some) 5 days and frequent transfers are necessary. The injection into the yolk sac retards the process of infection in the embryo.

13230 P

Phenomenon of Local Tissue Reactivity to Bacterial Filtrates Elicited in Heart and Femoral Artery.

C. Tedeschi* and N. C. Schlossmann.† (Introduced by G. Shwartzman.)

From the Laboratories of the Mount Sinai Hospital, New York City.

The following report deals with an attempt to reproduce the Shwartzman phenomenon of local tissue reactivity[1] in the heart and blood vessels. The experimental procedures were similar to those employed by others in various organs in the elicitation of this immunologic response. Stock rabbits of the average weight of 2 kg were used. "Agar washings" filtrate of 24-hour-old cultures of meningococcus (strain B group 3) were employed for preparatory and provocative injections.

Shwartzman Phenomenon in the Heart. Rabbits were given single intracardial injections of the filtrate directly into the heart muscle through the epilated intact chest wall. Others received single intravenous injections of the same filtrate. The protocols of these control experiments are omitted. It was found that a dose of 0.03 cc of the filtrate diluted 1:30 produced a negligible primary tissue reaction and thus could satisfactorily serve our purpose. Various concentrations of the intravenous provocative units were always injected in a volume of 1 cc per kg of body weight. In all experimental animals submitted to the double injections a myocardial necrotizing hemorrhagic lesion was the outstanding feature at the site of preparation. This effect appeared promptly following the intravenous injection. The degree of muscle necrosis and infiltration with polymorphonuclear leucocytes were subject to individual variation. Vascular injury found constantly consisted of various degrees of venous congestion, frequently very marked, and accompanied by hemorrhage and thrombosis (homogeneous plugs, hyaline in appearance). Pyknosis, rhexis and lysis of the nuclei of the leucocytes, only rarely observed in the controls, were so pronounced in the animals receiving the combined local and intravenous injections that this further served to delineate the unique features of the phenomenon. While acute parietal thrombo-endocarditis (Fig. 1)

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* Dazian Fellow for Medical Research.

† Theodor Escherich Fellow in Pathology.

[1] Shwartzman, G., *Phenomenon of Local Tissue Reactivity,* Paul B. Hoeber, New York, 1937.

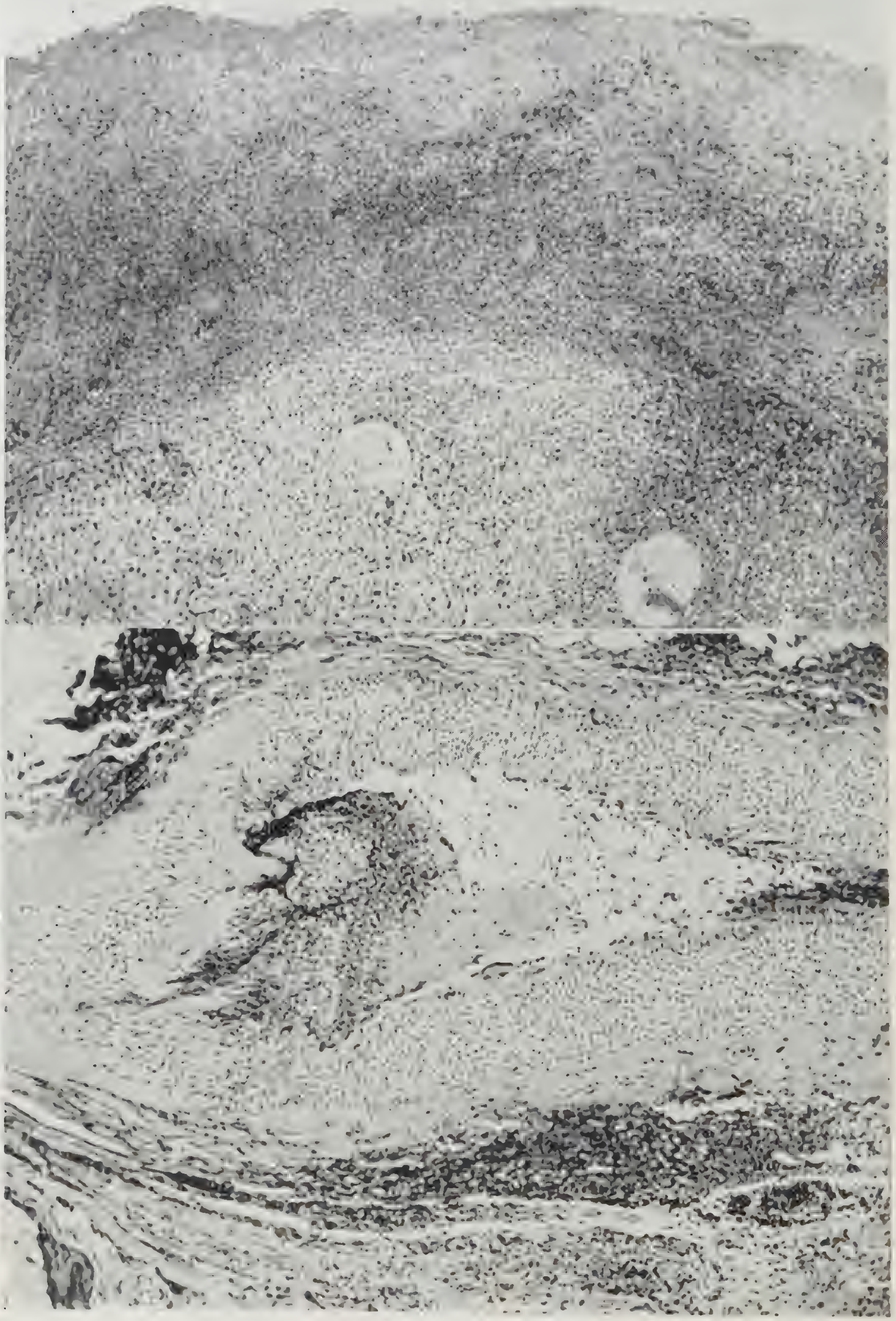

FIG. 1.
Microphotograph of parietal thrombo-endocarditis and mural thrombosis in femoral artery produced in rabbit at the site of local preparatory inoculation subsequent to intravenous injection of ''agar washings'' meningococcus filtrate. × 95.

was seen in 5 animals, valvulitis was noted only once. The latter finding must, therefore, be considered with reservation.

Shwartzman Phenomenon in the Femoral Artery. Again, the meningococcus filtrate in a dose of 0.03 cc of a dilution of 1:30 produced the same negligible primary tissue reaction as in the heart. Three rabbits were prepared peri-arterially. Three other rabbits received the preparatory injection into the lumen of the artery. All rabbits were injected intravenously 24 hours later with the same filtrate as above (1 cc per kg of filtrate, diluted 1:30). In each instance the reaction at the site of preparation was characterized by an outstanding necrotizing and hemorrhagic lesion. Overwhelming leucocytic infiltration and frequent luminal thrombosis were consistently found in both series (Fig. 1).

In 3 out of 4 animals the phenomenon was successfully elicited simultaneously in the heart and femoral artery.

Summary. To sum up, the Shwartzman phenomenon of local tissue reactivity was successfully produced in the heart and blood vessels by the use of "agar washings" filtrates of meningococcus. The hemorrhagic and necrotizing feature of the reaction together with altered vascular response at the sites of preparation were consistent findings.

13231 P

Smooth Muscle Effects of Carnosine.

DONALD SLAUGHTER, T. U. JOHNSON AND J. WESLEY GALES.
(Introduced by V. du Vigneaud.)

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Koinaroff[1] reported that carnosine, B-alanyl-l-histidine, produced a marked increase in the tonus of isolated rabbit intestine. McClintock and Hines[2] confirmed this observation and also noted that carnosine caused a similar increase in tonus and rhythmical contractions of the isolated guinea pig uterus. Contrary to this view is the work of McClosky, *et al.*,[3] who were unable to substantiate the reported oxytocic effect of carnosine on uterine strips.

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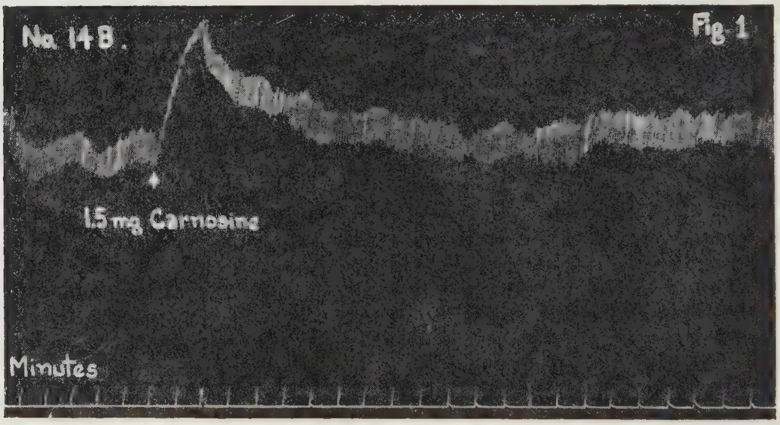
[1] Koinaroff, S. A., *Chem. Zentralblt.*, 1922, 636.

[2] McClintock, J. T., and Hines, H. M., PROC. SOC. EXP. BIOL. AND MED., 1924-25, **22**, 515.

In the light of the somewhat conflicting work on uterine strips, we thought it would be worthwhile to reinvestigate the smooth muscle action of carnosine.* In the present work, isolated monkey† intestine was used. Oxygenated Tyrode's solution served as a physiological bath for these experiments. The temperature was kept at 37.5°C. Forty-eight experiments using various doses of carnosine were performed. Fig. 1 illustrates the typical effect as noted with 1.5 mg of this drug in a 100 cc bath.

For a long time we have been interested in the fact that morphine causes a relaxation of the isolated intestine in various animals, but markedly stimulates the tone of the intact intestine. While many workers, Dreyer,[4] Gruber, *et al.*,[5] and others have reported a stimulating effect of morphine on isolated intestine, we have never



This illustrates the marked stimulant effect of carnosine on isolated monkey intestine. Time trace, minutes. Kymograph speed, 6 mm per minute. Bath, 100 cc.

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[3] McClosky, W. T., Miller, L. C., Hunt, Madison, and du Vigneaud, Vincent, PROC. SOC. EXP. BIOL. AND MED., 1937-38, **37**, 60.

* The carnosine used in this study was prepared and kindly furnished by Dr. Vincent du Vigneaud.

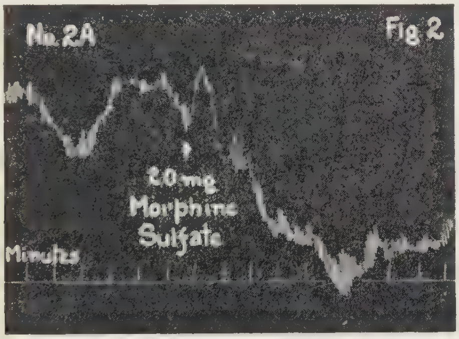
† Furnished by Dr. A. I. Folsom, Department of Urology, Baylor University Medical School. These were castrate female monkeys who had received repeated doses of testosterone propionate. Expert opinion given indicates that such treatment would not alter the activity of the intestine.

[4] Dreyer, N. B., *Arch. Internat. de Pharmacol. et de Therap.*, 1933, **45**, 397.

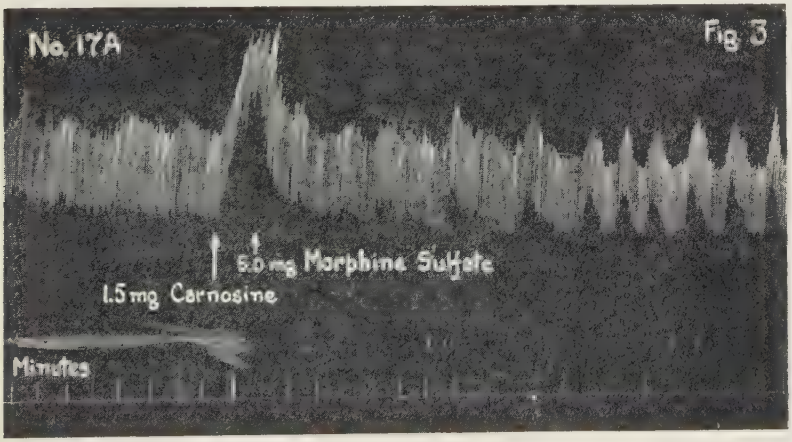
[5] Gruber, C. M., Brundage, J. T., De Note, Anthony, and Heiligman, Raymond, *J. Exp. Pharm. and Therap.*, 1935, **55**, 430.

consistently obtained such results. Rather, it has been our belief that in isolated tissue some necessary substance was lacking which prevented morphine from giving a typical response such as is seen in the intact animal. To test this hypothesis, 25 experiments were done on the isolated monkey intestine using morphine alone. Fig. 2 exemplifies the usual response; *i. e.,* relaxation. When carnosine was given previously, however, a large dose of morphine did not cause relaxation (Fig. 3).

Since the tissues available were not too extensive (10 monkeys), we hesitate to report a few suggestive results which seem to indicate that carnosine may have a cholinergic-like effect on the isolated intestine.



This tracing demonstrates the typical effect of morphine on isolated monkey intestine. Note the marked relaxation of intestinal tonus. Time trace, minutes. Kymograph speed, 6 mm per minute. Bath, 100 cc.



This tracing illustrates that morphine does not cause relaxation of isolated monkey intestine; that is, after carnosine has been given previously. Time trace, minutes. Kymograph speed, 6 mm per minute. Bath, 100 cc.

We feel that these results indicate that carnosine is a stimulant of isolated intestine and that it prevents the morphine relaxation effect on such tissues. Since morphine always increases the tone of the intact intestine, it may be that "carnosine lack" is the cause of the failure of such a response in isolated material. Studies to correlate the disappearance of carnosine with the effect of morphine on the isolated intestine are planned.

13232 P

Relation of Gastric Acidity to Alveolar Bone Resorption.

JULIUS BRECHNER AND W. D. ARMSTRONG.
From the Laboratory of Dental Research, University of Minnesota, Minneapolis, Minn.

The absorption of calcium occurs chiefly from the upper part of the small intestine, the reaction of whose contents has been shown[1] normally to be acid. Irving and Ferguson[2] found the absorption of calcium to be facilitated when solutions of calcium chloride, deposited directly in the intestine of dogs, were buffered so as to maintain an acid reaction. There is a direct relationship between pH of the intestinal contents and gastric free acidity[3] and with advancing age there is a reduction in gastric acidity of both sexes.[4] Clinical experience[5] indicates that periclasia, one feature of which is alveolar bone resorption, is a condition which rarely occurs before age 20 but increases markedly in incidence after age 30. The coincidence of approximately equal age distributions of cases of physiological hypochlorhydria, periclasia and senile osteoporosis suggests that some cases of alveolar bone resorption are due to deficient calcium absorption consequent upon the secretion of an amount of hydrochloric acid by the stomach insufficient to maintain the normal acidity of the intestinal contents.

Seventy-nine individuals were subjected to gastric analyses, using

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[1] Myers, F. J., and McClendon, J. F., *J. Biol. Chem.*, 1920, **41**, 187.

[2] Irving, L., and Ferguson, J., PROC. SOC. EXP. BIOL. AND MED., 1924-25, **22**, 527.

[3] Kearney, R. W., Comfort, M. W., and Osterberg, A. E., *J. Clin. Invest.*, 1941, **20**, 221.

[4] Vanzant, F., Alvarez, W. D., Eusterman, G. B., and Dunn, H. L., *Arch. Int. Med.*, 1932, **49**, 345.

[5] Brekhus, P. J., *J. Am. Dent. Assn.*, 1929, **16**, 2237.

50 cc of 7% alcohol as the test meal. The method of Bloomfield and Keefer[6] was used for the gastric analyses, according to which, the entire stomach contents were aspirated and all of the contents returned to the stomach except for a 10 cc sample. This procedure was repeated each 10 minutes for one hour or until the stomach was empty. The samples were separately titrated and the results calculated so as to express the average contents of free and total acidity in terms of milliequivalents of hydrochloric acid per liter. In those cases exhibiting achlorhydria to alcohol stimulation the analysis was repeated with histamine stimulation. The procedure was repeated with three persons several weeks after the first gastric expression with the result that the average difference of the duplicate determinations of free hydrochloric acid from their mean was 3.9%.

Full mouth roentgenographic examination revealed that 42 individuals manifested alveolar bone resorption in varying degree and that 37 persons (control group) showed no evidence of alveolar bone resorption. The results of the gastric analyses are shown in Table I, where the figures in parenthesis indicate the extreme variation of the results entering into each mean. The standard deviation of the means of the pooled results for the males and females are shown as the lowermost number in each column. The ratios of the difference of comparable means to the standard error of the difference of the means were respectively: mean free acid 5.0, total acid 4.7,

TABLE I.
Relation of Free and Total Gastric Acidity to Alveolar Resorption.

No. of cases and sex	Age, avg	Mean free acid M. Eq./liter	Mean height of free acidity M. Eq./liter	Mean total acid M. Eq./liter
		Control Group.		
16 M	28.6	32.5	46.0	46.0
	(16-42)	(4.7-60.3)	(8.2-81.2)	(13.0-76.8)
21 F	27.0	23.6	35.9	38.4
	(16-53)	(0.0-45.8)	(0.0-79.4)	(4.8-81.1)
37 M-F	27.0	27.4	40.2	41.7
	(16-53)	(0.0-60.3)	(0.0-81.2)	(4.8-81.1)
Standard deviation of aggregate group		14.50	19.25	19.09
		Alveolar Resorption Group.		
25 M	31.4	13.9	21.4	25.0
	(21-43)	(0.0-59.3)	(0.0-79.6)	(3.6-78.5)
17 F	32.6	10.3	16.2	20.9
	(19-51)	(0.0-23.8)	(0.0-42.0)	(3.0-38.5)
42 M-F	31.9	12.4	19.3	23.3
	(19-51)	(0.0-59.3)	(0.0-79.6)	(3.0-78.6)
Standard deviation of aggregate group		11.52	17.30	15.03

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[6] Bloomfield, A. L., and Keefer, G. S., *J. A. M. A.*, 1927, **88**, 707.

and height of acidity 5.0. The probabilities of the higher values of the means of the control group resulting from chance is, therefore, remote. Too few cases were available for valid comparison of the means for men and women separately but the individual values of means in the control group exceeded those in the alveolar resorption group by 83 to 134%.

13233

Diurnal Rhythm of Melanophore Hormone Secretion in the *Anolis* Pituitary.

H. RAHN AND F. ROSENDALE. (Introduced by B. H. Willier.)

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The *Anolis* lizard exhibits a striking color change by assuming a bright green color at night and usually turning a dark brown under the influence of light. This spectacular metachrosis is brought about by the concentration or dispersion of pigment in the melanophores which exposes or obscures respectively a green reflecting chromatophore layer and thus produces either a bright green or a dark brown animal. These dermal melanophores are directly controlled by the secretions of the pars intermedia of the pituitary gland (Kleinholz[1]). The removal of this region or of the whole pituitary induces a permanent pigment concentration and consequently a green animal, while injections of melanophore hormone produce pigment dispersion and a dark brown animal. Thus hormone release and inhibition are easily recognized in a normal animal by its striking effect upon the integument color.

In preliminary experiments these lizards exposed to continuous light on a white background at 22° and 40°C for as long as 4 days exhibited a tendency to turn green during the corresponding night period and dark brown during the day. (Anolis lizards were obtained from Ocala, Florida.) This suggested a diurnal rhythm which was further tested by placing one group of 13 and another of 11 lizards in constant darkness for 8 and 18 days respectively. Throughout this period the temperature remained within a 22-24°C range. Observations were made twice to 4 times within the 24-hour day and were spaced at least 4 hours apart.

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[1] Kleinholz, L. H., *Biol. Bull.*, 1935, **69**, 379; *J. Exp. Biol.*, 1938, **15**, 474.

Within 2 days after confinement to constant darkness it became evident that the animals exhibited a diurnal rhythm in their color response. During the solar night all animals turned green, while during the solar day up to 90% of them changed to brown. These periods of metachrosis commenced rather precisely at 6 A.M. and 4 P.M. and were completed within 2 hours. Fig. 1 shows the results of 8 days of observations on 13 animals in which the percentage of dark animals is plotted against the time of day. The observations on the other series of animals confined for 18 days to total darkness yielded similar results. In these tabulations an animal was considered dark when no more green was visible or, more precisely, when they assumed stage 3 or 4 of the color scale proposed by Kleinholz and Rahn.[2]

It will be seen from Fig. 1 that 10-15% of the animals did not turn dark during the solar day. Upon exposure to the observation light these lizards required on the average 1.6 minutes to turn dark, while during the solar night the average reaction time was 3.9 minutes. This suggests that even in these animals there is a diurnal change in the melanophore hormone level which is, however, not

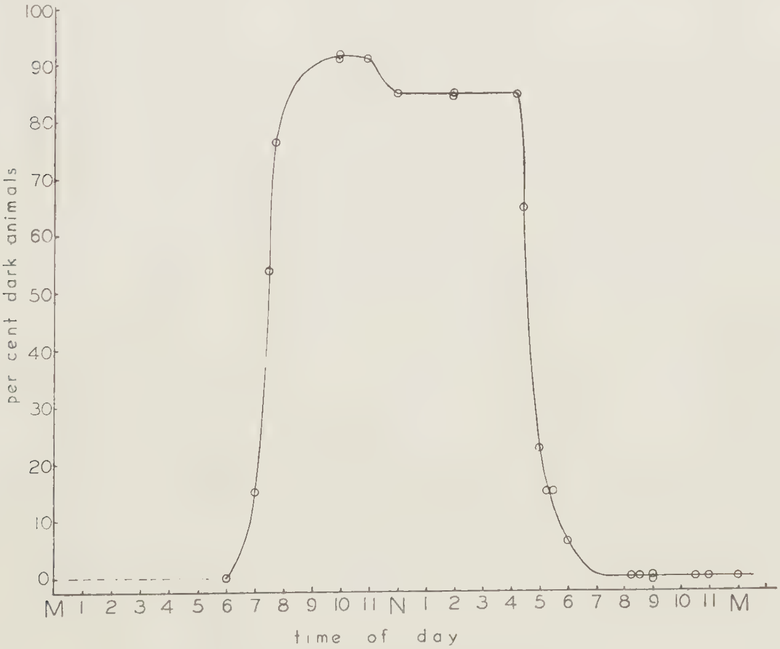

Fig. 1.
For explanation see text.

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[2] Kleinholz, L. H., and Rahn, H., *Anat. Rec.*, 1940, **76**, 157.

large enough to attain the melanophore threshold during the solar day.

Lizards exposed continuously for 6 days on a black background to 3 foot-candles did not exhibit a very strong color rhythm. On an average 98% of them remained dark during the solar day, but only 40% turned green between 7 P.M. and 8 A.M. These observations were further complicated by a high mortality among the animals, yet they suggest that even continuous exposure to light was not able entirely to suppress the secretion rhythm.

In control experiments 30 lizards were completely or partially (pars intermedia only) hypophysectomized and exposed to normal changes of night and day for as long as 3 weeks. In no case was darkening observed, all animals remaining bright green.

These observations then lead to the conclusion that the pars intermedia of the *Anolis* lizard pituitary exhibits a diurnal secretory cycle which is independent of light receptors, but is normally reinforced by the alternating day and night period. In the lamprey,[3] *Xenopus,*[4] and *Salamandra maculosa*[5] similar observations on a persistent melanophore rhythm in continuous darkness have been reported. In *Salamandra* a definite cycle exists for only a week, while in both this form and the lamprey continuous light disrupts the rhythm. In all these forms, especially *Xenopus,* the reported melanophore rhythm is very slight, never attaining the maximal states of concentration and dispersion as seen so strikingly in *Anolis.* It would be premature to speculate concerning the control mechanism of this cyclic secretion process were it not for the recent evidence of Etkin,[6] who finds the functional activity in the pars intermedia of *Rana* normally restrained, probably through the mediation of the hypothalamic-hypophyseal tract. For *Anolis* this suggests possibly an activity rhythm in the hypothalamic nuclei which controls the diurnal release of the melanophore hormone from the pars intermedia.

Summary. *Anolis* lizards which normally exhibit a different color response to day (brown) and night (green) were subjected to continuous light or darkness over a period from 6 to 18 days. Under the former condition a weak, but under the latter a very marked and precise diurnal color rhythm was exhibited for as long as 18 days. Hypophysectomized animals remained permanently green.

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[3] Young, J. Z., *J. Exp. Biol.*, 1935, **12**, 254.

[4] Hogben, L., and Slome, D., *Proc. Roy. Soc. B.*, 1931, **108**, 10.

[5] Pauli, W., *Z. wiss. Zool.*, 1926, **128**, 421.

[6] Etkin, W., *J. Exp. Zool.*, 1941, **86**, 113.

13234

Studies on the Cultivation of *Sp. gallinarum.*

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Since Noguchi's[1] classic experiments on the cultivation of spirochetes a number of workers have attempted to discover the conditions favorable to primary cultivation and continued subculture of this group of organisms. Most workers have failed to cultivate *Tr. pallida,* whereas several have reported successful results with the genus Spironema.[2-5] The shortcomings of the various media hitherto proposed are twofold: they are complicated and hence contain many variables; other workers have not always been able to repeat the cultivation with the same degree of success.

In connection with studies on fowl spirochetosis we have undertaken a systematic study of the nutritive requirements of this organism, studying each component in turn in order to establish to what extent it is required and, if required, its optimum concentration. The results of these experiments are summarized in this note.

The test of the suitability of a medium of given composition consisted in ascertaining by ultramicroscopic examination the density of growth and the viability of the spirochetes and also the ease with which passages could be carried on when minimal numbers of organisms (1 to 10 or 1 to 20 fields) were inoculated.

The medium finally obtained, which in our hands has given constant and repeatable results consists of the following ingredients:

1.	Basic salt solution*	1000.00 ml
	Proteose Difco peptone	4.00 g
	Dextrose	1.00 ”
	Sodium lactate	3.00 ”

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[1] Noguchi, H., *J. Exp. Med.*, 1912, **16**, 199.

[2] Kligler, I. J., and Robertson, O. H., *J. Exp. Med.*, 1922, **35**, 303.

[3] Marchoux, E., and Chorine, V., *Compt. rend. Soc. de Biol.*, 1931, **106**, 1125.

[4] Galloway, J. A., *Compt. rend. Soc. de Biol.*, 1926, **93**, 1074.

[5] Landauer, E., *Compt. rend. Acad. de Sc.*, 1931, **193**, 301.

* The salt solution was made up as follows:

NaCl	5.00 g
Na_2HPO_4	2.50 ”
KH_2PO_4	0.25 ”
$MgCl_2$	0.30 ”
H_2O	1000 ml

Thioglycollic acid	0.001 g

2. Before inoculation we add to each 10 cc of this medium 1.0 ml rabbit serum and 0.6 ml of sedimented chicken red cells resuspended in an equal volume of the basic salt solution.

Salt Mixture. The basic salt mixture can be either Tyrode solution or the special salt mixture given below in which the bicarbonate is replaced by phosphate, which is a more satisfactory buffer. To the salt or Tyrode solution traces of iron and manganese salts were added. The phosphates were added in the proportion necessary to give a pH 7.7-7.8.

Aerobiosis. From the work of Kligler and Robertson[2] and Marchoux and Chorine,[3] it was apparent that these organisms are aerobic but require a lower reduction potential for initial growth than do bacteria. At the outset we were able to show that 1 mg ascorbic acid per 10 cc of medium had a favorable effect on growth. Since, however, ascorbic acid is readily oxidized we subsequently used thioglycollic acid in the concentration given above with equally good results. It is possible to use twice the amount indicated but higher concentrations have an inhibitive effect.

pH. The optimal reaction for initial growth is pH 7.6-7.7. The end reaction in media in which good growth was obtained is about pH 6.8. Growth is good also in the same medium with an initial pH 7.3, but it is less abundant. At reaction of pH 6.6 the organisms remain viable but no growth occurs. The limiting zone of reaction is, therefore, quite narrow.

Nitrogen Requirements. In the various reports in the literature there are conflicting views regarding the need of peptone. Galloway[4] did not use peptone. Marchoux found that 0.2% Witte peptone improved growth in Galloway's medium. We tested Witte, Difco and Difco Proteose peptone, in various concentrations. The results showed that best growth was obtained in media containing 0.4% proteose peptone; 0.6% gave the same results; 0.8% gave somewhat poorer growth. A really inhibiting effect of peptone was not noted. These results suggest that a certain optimal concentration of the required amino-acids is essential. The amino-acids required by this organism is now being investigated.

Carbohydrate Requirements. Galloway[4] and Marchoux and Chorine[3] found that glycogen was essential for growth and the latter authors claim that 0.1% glucose inhibits growth. We have not been able to confirm either of these findings. Our results show that a combination of lactate and glucose is most favorable for growth, that in the presence of lactate higher concentrations of glucose up to

0.2% have no unfavorable effect and that in their presence glycogen proved an indifferent substance. It is questionable, therefore, whether the favorable effect of glycogen noted by the authors mentioned above is due to its function as a nutrient. As already noted the reaction of the medium changes during growth to the acid side although the amount of sugar consumed is apparently very small. The carbohydrate metabolism is now being studied.

Serum. Various sera were tested—chicken, rabbit, guinea pig, sheep, cow and rat. Rabbit serum gave the best results, guinea pig and cow, poorest. In the absence of serum, even though blood is added, there is no, or only slight, growth. Growth improves with increasing amounts of serum until 10.0% is reached. The factor in the serum essential for growth has not yet been established, but experiments made thus far suggest that it is heat labile. Serum heated at 86-90°C for half an hour is unfavorable for growth; initial growth is poor and viability of short duration. Inactivation of serum (56°C for half an hour) favors moderate initial growth in the absence of blood although the organisms are short lived; in active serum growth is sparse unless blood is added.

Blood Cells. Chicken red cells are essential for good growth and viability. The optimum amount is 0.6 cc of a 50% cell suspension to 10 cc of medium. Rabbit blood cells are also good, but less satisfactory than those of chicken. Transplants failed rapidly when red cells were excluded. It still remains to determine whether there is some factor in the red cell other than the hemoglobin that plays a rôle in growth.

Accessory Substances. Since growth in this, as in other media, does not approximate that of bacteria we tested the effect of the addition of accessory substances. To 10 cc of medium were added 30 γ and 3 γ of thiamine, 3 γ and 30 γ of riboflavin, 1.5 and 0.3 mg β-alanine singly and in combination without noticeable effect. Glutamine and pimelic acid were also without influence.

Liquid Paraffine. A layer of 1 to 2 cm of paraffine was found by all authors to be essential. The rôle of the paraffine is most probably that of regulator of the gas exchange and the maintenance of an optimum balance, because equally good growth was obtained when paraffine was eliminated and the tubes closed with rubber stoppers.

Conclusion. On the medium described above we have been able to cultivate *Sp. gallinarum* without difficulty. The cultures grow best at 37°C, but growth occurs also at 30°C and at 39°C; at 30°C growth is slower but the viability better than at 37°C, at 39°C the reverse is the case. The cultures reach optimum growth density in 4 to 5 days according to the size of the inoculum. If transferred to the ice box at this point they remain active for 10 days to 2 weeks. Transplants have been made as late as the 15th day of culture. One strain has been carried through 19 generations during a period of 150 days. The 18th subculture of this strain was still fully virulent for chickens and caused fatal infections.

13235

Action of Sulfamido Compounds upon *M. lysodeikticus* and Lytic and Bactericidal Activities of Lysozyme.

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In contrast to many antiseptics used in surgical practice, sulfanilamide and its derivatives do not interfere with the lytic action of bacteriophage. In view of the fact that lysozyme has certain similarities to bacteriophage, experiments were carried out to determine what effects these chemotherapeutic substances have upon the lytic and bactericidal activity of lysozyme. Furthermore, the effects of sulfanilamide upon the growth of *M. lysodeikticus* were studied. Finally, experiments were done to determine whether or not lysozyme and sulfamido compounds act synergistically, as do sulfamido compounds and other anti-microbial agents, *e. g.*, immune serums, bacteriophage and certain disinfectants.

As a source of lysozyme egg white powder* was used. A strain of *M. lysodeikticus* was obtained through the courtesy of Dr. Marshall L. Snyder, Ann Arbor, Michigan. Brain heart infusion broth (Difco) was used as culture medium. Physiological saline solution and citric acid-disodium phosphate buffer, as recommended for blood cultures by Nelson,[1] were also employed.

The strain of *M. lysodeikticus* was grown on plain agar slants at 37°C. Suspensions were prepared either in physiological saline solution, distilled water or buffer solution.

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* The egg white powder was kindly supplied by Stein, Hall & Company, Inc., New York, New York.

[1] Nelson, C. I., *J. Infect. Dis.*, 1940, **66**, 113.

Lysis of the organism was appraised grossly. Viability of the organisms was determined by seeding plain agar plates with a standard loopful of the material; the plates were incubated at 37°C for 72 hours.

The effect of sulfanilamide upon the growth of *M. lysodeikticus in vitro* was first investigated. Brain heart infusion broth containing sulfanilamide in concentrations ranging from 1 mg % to 1000 mg % as well as broth as a control (volume 5 cc) were seeded with 0.2 cc of a heavy suspension of *M. lysodeikticus* in one experiment, and with 0.2 cc of a light suspension in another. The tubes were incubated at 37°C for 48 hours. Growth was noted at various intervals. It was found that in a concentration of 1000 mg %, sulfanilamide completely prevented the growth of *M. lysodeikticus*, provided small numbers of organisms were used for seeding purposes. In concentrations of 10 mg % to 100 mg % this drug only slightly delayed the growth of the organism.

The effect of sulfanilamide, sulfapyridine and sulfathiazol on the lytic activity of lysozyme (egg white) toward *M. lysodeikticus* was then investigated. The drugs were dissolved and the organisms suspended in physiological saline solution, distilled water, or citric acid-disodium phosphate buffer (pH 7.2) solution. The latter was used in order to exclude interference of different pH values. The results of all experiments were essentially identical. A typical experiment follows:

Sulfanilamide in concentrations of 0.1 mg % to 1000 mg % and sulfapyridine in concentrations of 0.01 mg % to 100 mg % were mixed with egg white powder (100 mg %). Controls without sulfanilamide and sulfapyridine and controls without egg white were included. The mixtures (volume 4.0 cc) were kept for 5 minutes at 55°C in part I of the experiment and for 24 hours at 55°C in part II. Then a suspension of *M. lysodeikticus* (volume 0.2 cc) was added, and the test tubes were incubated at 55°C. Clearing was noted at various intervals. The experiment revealed that neither sulfanilamide, sulfapyridine nor sulfathiazol interfered with the lytic activity of egg white toward *M. lysodeikticus*, even if these drugs were allowed to act upon lysozyme for 24 hours at 55°C. Experiments carried out at 37°C revealed essentially the same findings.

These results, therefore, indicate that lysozyme belongs to that group of enzymes or enzyme-systems that are not inhibited by drugs of the type of sulfanilamide. Although the rôle of lysozyme as a defense factor against microbial infections is still a moot question, the demonstration that sulfamido compounds even in high concentration do not interfere with lysozyme activity *in vitro* gives indirect evidence that the administration of these chemotherapeutic substances probably does not affect its lytic or bactericidal activities *in vivo*.

In another series of experiments the susceptibility to the action of lysozyme of *M. lysodeikticus* that was previously exposed to the action of sulfamido compounds was determined. The experiments revealed that egg white powder caused lysis of the organisms irrespective of whether they were grown in the presence or absence of sulfanilamide.

The question arises as to whether or not sulfamido compounds and lysozyme act synergistically and together exert greater bactericidal effects than does either agent alone. A typical experiment follows: Egg white powder in concentrations of 100 mg %, 50 mg %, and 20 mg % in (a) buffer solution and in (b) buffer solutions containing sulfapyridine in a concentration of 100 mg %, was incubated at 55°C for 2 hours. At this state of the experiment cultures were made in order to test for sterility. Then a suspension of *M. lysodeikticus* in buffer solution was added. The tubes were incubated at 37°C for 2 weeks. Lysis and survival of the organisms were determined at various intervals. The experiment revealed that neither egg white in a concentration of 100 mg % or less nor sulfapyridine caused loss of viability of the organisms even after an incubation period of 15 days. In contrast, egg white in concentrations of 50 mg % and above used together with sulfapyridine killed the organisms after 8 days' incubation. Essentially the same results were obtained with sulfanilamide in a concentration of 1000 mg %. It may be concluded, therefore, that the combined use of sulfanilamide or sulfapyridine and lysozyme results in somewhat greater bactericidal effects than that of either compound alone.

Several experiments were designed in order to compare the action of sulfamido compounds upon lysozyme with other commonly used disinfectants. In concentrations that did not cause visible precipitation of egg white (lysozyme), phenol (1:100); bichloride of mercury (1:100,000); merthiolate (1:10,000); and zephiran (1:100) did not interfere with the lytic action of lysozyme. In contrast, tincture of iodine in concentrations that did not cause visible precipitation of egg white, definitely inhibited the lytic activity of lysozyme.

Summary. (1) Sulfanilamide in high concentration (1000 mg %) exerts bacteriostatic activity toward small numbers of *M.*

lysodeikticus. (2) Sulfanilamide in concentrations up to 1000 mg % and sulfapyridine and sulfathiazol in concentrations up to 100 mg % do not interfere with the lytic activity of lysozyme (egg white). (3) The combined use of lysozyme (egg white) and sulfamido compounds (sulfanilamide and sulfapyridine) exerts somewhat greater bactericidal effects upon *M. lysodeikticus* than either agent alone.

13236

II. Effects of Sulfanilamide and Sulfapyridine upon Experimental Streptococcal Infections.

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Having obtained a suitable method for determining the virulence of *Streptococcus hemolyticus* in small laboratory animals, we proceeded to study the therapeutic effect of sulfanilamide and sulfapyridine upon infection. The infection consisted of injecting 0.03 cc of an 18-hour serum broth culture intracerebrally. In the experiment on the effect of sulfanilamide upon hemolytic streptococcal infection, strain SA_{17} was used. Two different dilutions of organisms were given and for each dilution the animals were divided into 2 groups. One group received the treatment and the other group served as a control. Each group consisted of 6 white mice and 3 hamsters. In the study on the effect of sulfapyridine upon hemolytic streptococcal infection, strain 232 was used instead. The animals were similarly divided and similarly infected except that only white mice were used and the number of animals employed for each group was 8. In the case of sulfanilamide, 3 mg in 1 cc normal saline was given intraperitoneally 2, 6, 10, 14, 24, 32, 50, 72, 96, 120, and 144 hours after the infection. The control group received parallel injections of 1 cc of normal saline each time. In the case of sulfapyridine, the drug was given by the oral route. The drug was suspended in a mixture containing one part of mucilage of tragacanth and one part of distilled water. The dosage was ½ cc containing 30 mg of the drug. The first treatment was given at the time of infection and subsequently 7, 24, 48, and 72 hours after the infection. The controls received similar amounts of diluted mucilage of tragacanth at the same time. All the animals were kept under observation for 2

TABLE I.
Effects of Sulfanilamide upon Intracerebral Route Infection with Strain SA_{17}.

No. org. inj.	2,660		266	
Treatment	Treated	Control	Treated	Control
Animals				
White mice	+3	1	S	2
	S	2	S	2
	S	2	S	3
	S	2	S	3
	S	2	S	3
	S	3	S	4
Hamsters	S	1	S	1
	S	1	S	2
	S	1	S	2

TABLE II.
Effects of Sulfapyridine upon Intracerebral Route Infection with Strain 232.

No. org. inj.	690,000		69,000	
Treatment	Treated	Control	Treated	Control
Animals				
White mice	+2	1	S	1
	3	1	S	1
	S	1	S	1
	S	1	S	1
	S	1	S	1
	S	1	S	1
	S	2	S	2
	S	2	S	2

weeks. Those that died were immediately autopsied and cultures from the brain were made. In all the autopsied cases the cultures were positive except those in the group treated with sulfapyridine, which showed no growth. The results are shown in Tables I and II.

Knowing that both sulfanilamide and sulfapyridine have good therapeutic effect on hemolytic streptococcal infection, we made a comparative study. The infection consisted of injecting 0.03 cc of an 18-hour serum broth culture intracerebrally. Animals were divided into 3 groups. The first group received sulfapyridine, the second group received sulfanilamide, while the third group received mucilage of tragacanth 1:3 dilution to serve as a control. Each group consisted of 9 white mice weighing from 16-18 g. For each group 3 different dilutions of organisms were given and for each dilution 3 animals were used. Sulfanilamide or sulfapyridine was suspended in a mixture containing one part of mucilage of tragacanth and 2 parts of distilled water and given by the oral route. The dosage was 0.5 cc containing 7.5 mg of the drug. The first treatment was given at the time of infection and subsequently 3, 7, 24, 32, and 48 hours after the infection. The results are shown in

TABLE III.
Comparative Therapeutic Effects with Small and Large Doses of Sulfapyridine and Sulfanilamide upon Intracerebral Route Infection with Strain 232 in White Mice.

Treatment	mg per dose	No. org. injected		
		1,300,000	130,000	13,000
Sulfapyridine	7.5	*4,S,S	S,S,S	S,S,S
	30	S,S,S	S,S,S	S,S,S
Sulfanilamide	7.5	1,S,S	S,S,S	S,S,S
	30	2,2,3	2,S,S	S,S,S
Control for	7.5	2,2,2	2,2,3	2,3,3
	30	2,2,3	2,2,3	2,3,5

*Each numeral represents days of survival of the animal after the infection and "S" represents the survival of the animals for 14 days.

Table III. All the control animals died within 3 days after the infection, while in the group receiving sulfanilamide or sulfapyridine there was only one death each. Postmortem cultures of the brain in the control group showed numerous colonies of *Streptococcus hemolyticus.* In the group receiving sulfanilamide, the brain culture of the animal that died yielded only 4 colonies while in the sulfapyridine-treated group the culture of the brain of the dead animal was sterile. The above experiment shows clearly that both drugs gave about the same degree of protection when given in doses as small as 7.5 mg. A similar experiment was performed with a larger dose on both drugs to study their toxic effect. The dosage was 0.5 cc containing 30 mg of the drug, and the treatment was given at the time of infection and subsequently 7 hours and one and 2 days after the infection. The results are shown in Table III. All the animals in the control group died, but none succumbed in the sulfapyridine-treated group. In the group receiving sulfanilamide 77% of the animals developed toxic symptoms in the form of paralysis of all 4 limbs. The paralysis occurred within 20 minutes after the administration of the drug. Some animals developed the symptom following the first administration, while some following the second. The paralysis gradually disappeared 4-6 hours later and reappeared after subsequent administrations of the drug. There were 4 deaths in the sulfanilamide-treated group which were apparently due to the toxicity of the drug. There was no toxic symptom in the sulfapyridine-treated group.

Conclusion. The method here presented appears satisfactory for chemotherapeutic investigation. The beneficial therapeutic effect of both sulfanilamide and sulfapyridine on hemolytic streptococcal infection in white mice and hamsters is presented. Sulfapyridine is definitely superior to sulfanilamide in that it is less toxic. A very large dose of sulfapyridine can be given at one time to sterilize the infection without producing any toxic symptoms. These experiments also indicated the prompt penetration of the drug into the brain tissue.

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Relation of Water Metabolism to Porphyrin Incrustations in Pantothenic Acid-Deficient Rats.

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It had previously been observed that the material which accumulated on the fur and noses of pantothenic acid-deficient rats exhibited a red fluorescence. This symptom had been erroneously described as "nose bleed"[1] and "blood-caked whiskers".[2] This fluorescent material contained large amounts of porphyrin, and the chemical tests for blood were negative.[3, 4] It was clearly demonstrated by an ablation experiment that the Harderian (lacrimal) gland is the source of this porphyrin secretion.[5]

During the preliminary preparation for the above experiment, it was observed that the water bottles in 2 of the cages had failed to deliver water. There were six 21-day-old albino rats in the 2 glass cages. Thick incrustations of fluorescent material were observed on the noses and near the medial angles of the eyes and smeared on the fur of all 6 rats. The other 18 rats with abundant water did not show this. Thus within a period of 3 days normal rats deprived of water developed what appeared to be one of the symptoms of pantothenic acid deficiency. When the rats were given water, they drank and then began to wash the incrusted material off. In less than one hour, they appeared to be as clean as the other 18 rats.

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* Rockefeller Fellow 1940-41 on year's leave of absence from the Department of Anatomy, University of Maryland School of Medicine, Baltimore, Maryland.

[1] Daft, F. S., Sebrell, W. H., Babcock, S. H., Jr., and Jukes, T. H., *Pub. Health Rep.*, 1940, **55**, 1333.

[2] Unna, K., *J. Nutrition*, 1940, **20**, 565.

[3] Chick, H., Macrae, T. F., and Worden, A. H., *Biochem. J.*, 1940, **34**, 580.

[4] McElroy, L. W., Salomon, K., Figge, F. H. J., and Cowgill, G. R., *Science*, in press.

[5] Figge, F. H. J., and Salomon, K., submitted for publication.

A relationship between water deprivation and pantothenic acid deficiency was suggested by the fact that both conditions had resulted in identical accumulations of red fluorescent material. Observations in other experiments supported this possibility.[3] Accordingly, the present experiment was conducted to study more carefully the relationship between various degrees of partial dehydration and the accumulation of the fluorescent incrustations on the fur and noses of rats.

Fourteen young albino rats (7 male, 7 female) were kept in individual cages and were given unlimited food (Tioga Dog Pellets) throughout the experimental period. Four of the animals were used as controls. During a 4-day preëxperimental period and during the experiment, the controls were allowed all the water they would drink, the quantity being measured daily. These values and the data of Richter[6] were used to calculate the normal daily water intake of rats. Of the remaining 10 animals, 4 were given 50%, four 37.5%, and two 25% of the normal water intake as calculated for rats of their age and size (12 cc/100 g body weight). The porphyrin accumulation, weight, and water ration or intake were recorded daily for all the animals.

By the end of the first week, 8 of the 10 water-limited animals had developed fluorescent porphyrin-containing incrustations around their noses. Less frequently, similar incrustations were observed on the eyelids and forepaws. There was little correlation between the amount of water given the animals and the degree of response except that there was a tendency for the animals with less water to develop the condition more rapidly than those with higher water intake. The incrustations were not present at all times, because the animals would either wash or rub them off during periods of increased activity.

During the second and third weeks, the water rations were variously manipulated (either increased or decreased). When the animals were maintained on less than 25% of normal intake or when completely deprived of water, the accumulation of porphyrin ceased, presumably because its secretion stopped. Porphyrin secretion and its consequent accumulation reappeared when the water ration was subsequently increased and disappeared as the normal body weight was approached. The increase in body weight was interpreted as indicating a return to the normal degree of hydration.

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[6] Richter, C. P., and Brailey, M. E., *Proc. Nat. Acad. Sciences,* 1929, **15**, 570.

At the end of the third week of the experiment, all the animals were sacrificed and the Harderian glands were examined under ultra-violet light. The glands were normal in all but 3 of the experimental animals. In the 2 rats in which porphyrin accumulation had been very slight or absent, the Harderian glands were found to be abnormal. In one of these rats, both glands were abnormally small; and in the other, one gland was small and almost non-fluorescent. However, in the third rat in which one gland was likewise small and non-fluorescent, porphyrin incrustation was similar to that observed in water-limited rats with normal glands.

Summary. Porphyrin incrustations on nose and fur, the most conspicuous symptom of pantothenic acid deficiency in albino rats, has been induced by partial dehydration of normal rats. This indicates that pantothenic acid may be involved in the regulation of water metabolism.

13238

Effect of Antigenic Material from *Eberthella typhosa* upon Migration of Guinea Pig Leucocytes.

H. R. MORGAN* AND H. C. UPHAM. (Introduced by T. D. Beckwith.)

From the Department of Bacteriology, University of California, Los Angeles.

Recent work by one of the authors (Morgan)[1, 2] demonstrated that an experimental leucopenia could be produced in rabbits by the intravenous injection of a protein-free antigen derived from *Eberthella typhosa.* Because of this property of the antigen it was desired to determine whether the material would exert a positive or negative chemotaxis towards polymorphonuclear leucocytes.

The technic employed in these experiments was that of Chambers and Grand.[3] It utilizes a small capillary tube filled with the material to be tested and one end of this tube is sealed with beeswax. This capillary is embedded in a drop of normal guinea pig plasma with its open end near a small fragment of normal adult guinea pig spleen. (The plasma was prepared with 1:1000 solution of heparin.)

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* Part of this work was carried out under the award of the Taussig Traveling Fellowship of the University of California.

[1] Morgan, H. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 529.

[2] Morgan, H. R., *J. Immunol.,* in press.

[3] Chambers, R., and Grand, C. G., *J. Cell. and Comp. Phys.,* 1936, **8**, 1.

After a few hours' incubation at 37°C the leucocytes begin to migrate from the fragment of spleen, and then, depending upon the properties of the material in the capillary tube which lies at right angles to their path, they either turn into the tube or avoid its open end.

The substances tested were (1) cells of *Staphylococcus albus* to serve as a control and (2) *E. typhosa.* Each of these was washed 3 times and suspended in Ringer's solution. The third was an antigenic extract of *E. typhosa* cultured in a synthetic medium and prepared as described by Morgan.[1, 2]

Twelve or more satisfactory preparations were made with each material and observed at intervals after 3 to 24 hours' incubation. The results of these tests, in brief, were as follows:

(a) With *Staph. albus,* which served as a control, the migration of the leucocytes from the splenic fragment was very pronounced, especially to the area about the point of the capillary tube. The leucocytes entered the tube in all preparations, and in most cases completely filled its lumen.

(b) With *E. typhosa,* the total migration of leucocytes from the splenic fragment appeared to be somewhat inhibited. The number of leucocytes reaching the tube was much smaller than with *Staph. albus* and but few of them entered the tube.

(c) With the antigen prepared from *E. typhosa,* the migration of leucocytes from the tissue appeared to be much repressed and few, if any, leucocytes invaded the capillary tube.

The foregoing results showed that although washed typhoid cells exerted a rather weak positive chemotaxis for the leucocytes, the antigenic extract of typhoid cells had a negative chemotactic effect. *Staphylococcus albus,* which gave strongly positive chemotaxis, constituted a useful control for the experiment.

It might be suggested that the release of this protein-free antigenic material from organisms of *E. typhosa* present in the lesions of the infected host may be a factor in the observed lack of infiltration of polymorphonuclear leucocytes into these areas in typhoid fever.

13239 P

Virus-Inactivating Capacity of Nasal Secretions in the Acute and Convalescent Stages of Influenza.*

THOMAS FRANCIS, JR., AND I. J. BRIGHTMAN.

From the Department of Bacteriology, New York University College of Medicine.

In an earlier publication[1] it was reported that the nasal secretions of human individuals possessed the capacity to inactivate influenza virus, Type A. It was noted that considerable variation existed among individuals. Burnet, Lush and Jackson,[2] studying material similarly obtained, observed no significant differences in the virus-inactivating potency of secretions of different individuals. Furthermore, they observed similar effects upon different viruses. They suggested, in consequence, that the virus-inactivating substance is enzymatic in nature.

On the other hand, studies conducted in this laboratory have indicated strongly that the agent responsible for the inactivation of influenza virus is an antibody and possesses many of the characteristics ordinarily attributed to antibody.[3] In a given individual, the content of virus-inactivating substance in the nasal secretions is fairly constant and uninfluenced by infections such as the common cold. Because of the similarity to antibody, it was of interest to ascertain what effect specific infection with influenza virus might exert.

During the past winter a widespread epidemic of influenza due, according to our observations, to influenza virus, Type A, was encountered. From 10 subjects, during the first 3 days of illness and again in the third week after onset, nasal secretions and samples of blood serum were obtained. These were then tested at the same time for their capacity to protect mice against infection with 1000 M.L.D. of PR8 strain of influenza virus, Type A, by means of the neutralization test. Four-fold serial dilutions of serum, ranging in final concentration from 1:4 to 1:256; two-fold serial dilutions of nasal secretions, ranging in final concentration from 1:2 to 1:16 were used. Three mice were inoculated intranasally with each serum-

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* This study was conducted under a grant from the International Health Division of the Rockefeller Foundation.

[1] Francis, T., Jr., *Science*, 1940, **91**, 198.

[2] Burnet, F. M., Lush, D., and Jackson, A. V., *Brit. J. Exp. Path.*, 1939, **20**, 377.

[3] Francis, T., Jr., *Problems and Trends in Virus Research*, University of Pennsylvania Press, Philadelphia, 1941, 41.

TABLE I.
Neutralizing Capacity of Nasal Secretions and Serum Taken During Influenza and in Convalescence.

	Neutralizing titer			
	Nasal secretions		Serum	
Patient	Acute	Conv.	Acute	Conv.
F	0	12	0	128
S	0	20	92	370
D	0	32	23	512+
Sm	0	20	32	178
B	0	6	12	370
E	5	20	32	128
G	5	32+	32	256+
L	10	12	8	32
W	0	32+	8	256+
Z	5	32+	8	512+

virus mixture. The animals were observed for 10 days, the day of each death recorded, and all survivors were sacrificed and their lungs examined for typical pulmonary lesions. The endpoint of each titration was estimated by the method of Reed and Muench.[4] Results of these procedures are presented in Table I.

It will be seen that in 9 of 10 instances a sharp rise in the capacity of nasal secretions to inactivate the virus occurred during convalescence. A simultaneous rise in circulating antibodies was also noted. It was of interest to note that all of these patients were over 50 years of age, but possessed little or no virus-inactivating substance at the time of the acute illness. This would tend to emphasize the possibility previously suggested that only those individuals essentially devoid of inactivating substance in their nasal secretions need be considered susceptible to infection.[5] The results of the tests serve also to support the evidence that the inactivating agent is antibody, in these instances apparently developed as a result of specific infection.

Summary. In 9 of 10 human individuals, natural infection with virus of influenza, Type A, was followed by the development of, or a sharp increase in, the capacity of their nasal secretions to inactivate the virus. Although not numerically parallel, increases in neutralizing antibodies of the blood were also observed. These results add further support to the earlier conclusion that virus-inactivating substance in nasal secretions possesses the characteristics of antibody.

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[4] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

[5] Francis, T., Jr., *Bull. N. Y. Acad. Med.*, 1941, **17**, 268.

13240

Rôle of Para-Aminobenzoic Acid in Vitamin B-Complex Studies with Mice.*

GUSTAV J. MARTIN AND S. ANSBACHER.

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Shortly after Rubbo and Gillespie[1] published the isolation of p-aminobenzoic acid as the benzoyl derivative from yeast, one of us (S.A.)[2] reported that this aromatic amine has vitamin properties and should be classified as a factor of the vitamin B-Complex. Its anti-gray-hair activity was first demonstrated in the nutritional achromotrichia of the rat[2] and subsequently in the hydroquinone achromotrichia of the mouse.[3] Our more recent studies showed that it has a pronounced influence on tyrosinase activity[4] and is capable of modifying enzymatic melanin formation.[5] Subsequently Lipmann[6] suggested its possible rôle in pigmentation processes. Emerson[7] found that it cannot replace pantothenic acid and Sure[8] confirmed its vitamin character. He observed, namely, it to be identical in its effects with vitamin B_x which is necessary for lactation and reproduction. Incidentally, the anti-gray-hair vitamin was also named vitamin B_x by Lunde and Kringstad.[9] Finally, Lampen and Peterson[10] presented data showing p-aminobenzoic acid to be a typical B-vitamin, namely, a growth factor for certain organisms, and Sieve[11] described its beneficial effect in human† achromotrichia.

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* Presented in part at Atlantic City, N. J., on September 11, 1941 (102nd Meet. of Am. Chem. Soc., Div. of Biol. Chem.).

[1] Rubbo, S. D., and Gillespie, J. M., *Nature*, 1940, **146**, 838.

[2] Ansbacher, S., *Science*, 1941, **93**, 164.

[3] Martin, G. J., and Ansbacher, S., *J. Biol. Chem.*, 1941, **138**, 441.

[4] Wisansky, W. A., Martin, G. J., and Ansbacher, S., *J. Am. Chem. Soc.*, 1941, **63**, 1771.

[5] Martin, G. J., Wisansky, W. A., and Ansbacher, S., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 26.

[6] Lipmann, F., *J. Biol. Chem.*, 1941, **139**, 977.

[7] Emerson, G. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 448.

[8] Sure, B., *Science*, 1941, **94**, 167.

† In clinical studies of asthma, one of us (S. A.) found that many asthmatics are greatly benefited by the oral administration of 100 mg of p-aminobenzoic acid t.i.d.

[9] Lunde, G., and Kringstad, H., *Norsk Pelsdyrblad*, 1939, **13**, 500.

[10] Lampen, J. O., and Peterson, W. H., *J. Am. Chem. Soc.*, 1941, **63**, 2283.

[11] Sieve, B. F., *Science*, 1941, **94**, 257.

In the present communication we wish to report on the rôle of p-aminobenzoic acid in nutritional investigations with mice.

Black mice (Rockland Strain) were divided into 2 groups of 75 animals each, one of which was fed a basal diet (casein, 18; cerelose, 67; salts, 4; cod liver oil, 2; butter fat, 9) supplemented daily with 7 of the B-complex vitamins, namely, 25 γ of thiamine hydrochloride, 50 γ of riboflavin, 25 γ of pyridoxine hydrochloride, 500 γ of nicotinic acid, 1000 γ of choline chloride, 250 γ of calcium pantothenate, and 500 γ of inositol. Graying of the fur was noted in 75% of these animals within a period of from 2 to 4 weeks. The second group received daily 250 γ of p-aminobenzoic acid in addition to the 7 vitamins and the hair of all the animals remained black during the 9-week test period. Apparently, p-aminobenzoic acid has the same effect in mice as Labco Rice Polish Factor II, the chromotrichial potency of which has already been reported.[12]

The rôle of p-aminobenzoic acid in the nutrition of the mouse is further demonstrated by the data of Table I. When mice were fed the basal diet supplemented with the 8 B-complex vitamins, no growth, no alopecia and no achromotrichia was observed until death which occurred in the 75 animals used in from 8 to 13 weeks. When calcium pantothenate was omitted in the diet of another lot of 75 mice, a pantothenate-deficiency developed in about 80% of the animals, the symptomatology of which was identical with the one recently described by Woolley.[13] When the lipotropic[14] and

TABLE I.
Pantothenate-deficiency, Achromotrichia and Alopecia.

Complements to basal diet supplemented by 5* B-complex vitamins			
Calcium pantothenate γ	Inositol γ	p-Aminobenzoic acid γ	Symptoms
none	none	none	pantothenate-deficiency
”	500	”	” ”
”	none	250	” ”
”	500	250	” ”
250	none	none	achromotrichia
250	500	”	”
250	none	250	alopecia
250	500	250	none

*Thiamine hydrochloride (25γ), riboflavin (50γ), pyridoxine hydrochloride (25γ), nicotinic acid (500γ), choline chloride (1000γ).

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[12] Martin, G. J., *Science*, 1941, **93**, 422.

[13] Woolley, D. W., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 565.

[14] Gavin, G., and McHenry, E. W., *J. Biol. Chem.*, 1941, **139**, 485.

spectacled-eye[15] factor inositol was omitted in the diet of another group of 75 mice, the typical[16, 17] alopecia appeared in about 80% of the animals. However, when inositol and p-aminobenzoic acid were omitted, the inositol-deficiency did not occur.[12] Since the addition of p-aminobenzoic acid to a heated vitamin K-deficient diet was observed[18] to result in an earlier incidence of avitaminosis, suggesting an inhibition of bacterial vitamin synthesis by the aromatic amine, it may be that inositol deficiency in mice on rations containing the amine is attributable to a bactericidal or bacteriostatic action of p-aminobenzoic acid. The report of Spink and Jermsta[19] "that the smaller concentrations of p-aminobenzoic acid in combination with a sulfonamide compound often appeared to inhibit growth more than was obtained with the sulfonamide compound alone," as well as preliminary bacteriological studies seem to substantiate this hypothesis.

Summary. Para-aminobenzoic acid has anti-gray-hair activity in the nutritional achromotrichia of the mouse. It is a dietary factor necessary for the demonstration of the anti-alopecia effect of inositol.

13241

Accelerated Hair Growth in the Rat After Adrenalectomy Cannot be Attributed to the Thyroid.

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From the Biological Laboratory, Hamilton College.

It is difficult to provide an explanation for the rapid hair growth following adrenalectomy in the albino rat since the function or functions of the cortical portion of the adrenal are not well understood (previous experiments[1] indicate that it is the cortex which is involved). This unusual growth upon adrenalectomy might be due to the thyroid or thyroxin as the following suggests: Thyroxin administration accelerates hair growth in the adrenalectomized rat;[1] the thyroid frequently hypertrophies and the appetite, glossiness of the fur, metabolism, and body temperature of rabbits often increase upon adrenalectomy;[2] the metabolism of the abdominal muscle of mice,[3] and the metabolism and body temperature of rats, cats and dogs[4] increase after adrenalectomy, but no increase is noted when both adrenals and thyroids are removed.

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[15] Pavcek, P. L., and Baum, H. M., *Science*, 1941, **93**, 502.

[16] Woolley, D. W., *J. Biol. Chem.*, 1940, **136**, 113; 1941, **139**, 29; *Science*, 1940, **92**, 384.

[17] Norris, E. R., and Hauschildt, J., *Science*, 1940, **92**, 316.

[18] Ansbacher, S., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 421.

[19] Spink, W. W., and Jermsta, J., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 395.

[1] Butcher, E. O., *Am. J. Physiol.*, 1937, **120**, 427.

On the other hand since activity in the hair follicle often starts within 48 hours after adrenalectomy in the rat,[5] it seems highly improbable that the thyroid can hypertrophy so quickly and induce the growth. In this study experiments have been undertaken to determine if adrenalectomy induces hair growth via the thyroid.

Procedure and Results. Underfed albino rats* with the hair removed from their backs were thyroidectomized and adrenalectomized when 36 days old (given 1% NaCl solution as drinking water after adrenalectomy). These animals produced visible hair on their backs in 8 or 9 days after the operation or as soon as litter mates which were only adrenalectomized. Rats which were thyroidectomized at the age of 36 days and then adrenalectomized 7 to 10 days later, likewise, produced hair apparent externally in 8 or 9 days after adrenalectomy. After the hair was growing well, the thyroidectomized animals were killed and the region around and including the larynx was fixed, sectioned in paraffin, and stained to see if all of the thyroid had been completely removed. In 13 of the 15 cases serial sections presented confirmatory evidence that thyroidectomy had been complete. Furthermore, no differences were detected between histological sections of the thyroids of underfed adrenalectomized rats and underfed litter mates.

It might be thought that thyroxin which is in the body at the time of adrenalectomy may induce activity in the hair follicles after adrenalectomy. To reduce the thyroxin in the body, underfed rats were thyroidectomized and 3 weeks later were adrenalectomized. The hair appeared externally in 8 or 9 days after the adrenalectomy which shows that thyroxin is in all probability not responsible for the immediate growth after adrenalectomy.

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[2] Marine, D., and Bauman, E. J., *Am. J. Physiol.*, 1921, **57**, 135.

[3] Davis, J. E., and Hastings, A. B., *Am. J. Physiol.*, 1933, **105**, 110.

[4] Grollman, A., *The Adrenals*, The Williams and Wilkins Co., 1936, p. 206.

[5] Butcher, E. O., and Richards, R. A., *Endocrinology*, 1939, **25**, 787.

* Rats were weaned and isolated at the age of 22 days, and thereafter fed finely ground Purina dog chow, usually 2 g in the morning and 2 g in the evening or just enough to prevent a loss in body weight. If the hair is removed from the backs of such underfed rats by shaving or by using sodium sulphide, the hair fails to grow back for long periods.[6]

[6] Butcher, E. O., *J. Nutrition*, 1939, **17**, 151.

Summary. When the hair is removed from the backs of underfed rats it does not grow externally for long periods. Upon adrenalectomy of such underfed rats, the hair grows on their backs in 8 or 9 days. Histological sections show no increased activity of the thyroid in such adrenalectomized animals. When underfed individuals are thyroidectomized one to 21 days previous to adrenalectomy, the hair, likewise, grows in 8 or 9 days after removal of the adrenals. There is, therefore, no evidence that the thyroid is responsible in any way for the rapid growth of hair in the rat following adrenalectomy.

13242

The Nature of Sulfanilamide Inhibition.*

ORVILLE WYSS. (Introduced by N. Kopeloff.)

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The mechanism of the action of the sulfonamides and the nature of the inhibition of their bactericidal effect by a number of compounds has not been clearly demonstrated. The discovery of the anti-sulfanilamide effect of p-amino benzoic acid by Woods[1] has opened a new approach to the problem. Fildes[2] explained this action as the inactivation by the sulfonamide of an essential enzymic grouping involving in some way p-amino benzoic acid. Support for this thesis was advanced when Rubbo and Gillespie[3] showed the compound to be a growth factor for *Cl. acetobutylicum.* They also showed that large amounts of sulfanilamide (23,000 mols. per mol. of p-amino benzoic acid) would prevent the growth occasioned by the presence of the growth factor. Thus it appears that p-amino benzoic acid may be an essential metabolite, the normal metabolism of which is disturbed by the presence of sulfanilamide.

The competitive nature of the actions of the sulfonamides and p-amino benzoic acid has been discussed by Lockwood.[4] Actual experimental proof of the competition has not been presented. Such proof can be obtained from the following mathematical considerations. If the following equations be assumed:

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* The author is indebted to Dr. F. C. Schmelkes for comments and suggestions.

[1] Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

[2] Fildes, P., *Lancet*, 1940, **1**, 955.

[3] Rubbo, S. D., and Gillespie, J. M., *Nature*, 1940, **146**, 838.

[4] Lockwood, J. S., *Surg., Gynecol. and Obstet.*, 1941, **72**, 307.

$$E + S \rightleftarrows E\,S \rightarrow E + P \tag{1}$$
$$E + I \rightleftarrows E\,I \text{ (inactive)} \tag{2}$$

the equations below can be derived.[5]

In the absence of inhibitor

$$\frac{1}{v} = \frac{1}{V}\,[K_s]\ 1/(S) + \frac{1}{V} \tag{3}$$

In the presence of inhibitor

$$\frac{1}{v_i} = \frac{1}{V}\left[K_s + \frac{K_s\,(I)}{K_i}\right]\frac{1}{(S)} + \frac{1}{V} \tag{4}$$

where

v = velocity of reaction in absence of inhibitor.
v_i = velocity of reaction in presence of inhibitor.
(S) = concentration of substrate.
(I) = concentration of inhibitor.
(E) = concentration of enzyme.
P = product of reaction (usually the measured quantity).
V = maximum velocity when enzyme is saturated with substrate.
K_s = dissociation constant of enzyme-substrate complex.
K_i = dissociation constant of enzyme-inhibitor complex.

Equations (1) and (2) have been manipulated to produce equations (3) and (4) because these permit the physical constants to be determined from experimental data. It can be seen that if the assumptions are correct (*i. e.*, if inhibition of the competitive type prevails) then, when the reciprocal of the velocity of the reaction is plotted against the reciprocal of the substrate concentration a straight line will result with an intercept of $1/V$ and a slope of $1/V\ [K_s]$ in the absence of an inhibitor and a slope of $1/V\left[K_s + \frac{K\ (I)}{K_i}\right]$ in its presence. Therefore the presence of an inhibitor will not affect the intercept of the line in such a plot but it will increase the slope by the amount $\frac{1}{V} \cdot \frac{K_s\,(I)}{K_i}$.

Experimental. A synthetic medium[6] was inoculated with 2,000,000 *E. coli* per ml and dispensed into test tubes calibrated for light transmission. The substrate p-amino benzoic acid and the inhibitor sulfanilamide were added and the cultures were incubated at 38°C. At intervals the turbidity was measured in a Hellige electrophotometer using a 660 millimicron filter which previous experiments had shown to give turbidity readings directly proportional to the number of organisms in the range of these experiments. Typical readings from the third to the seventh hour—the period of logarithmic growth—are recorded in Table I. It will be observed that p-amino benzoic acid in itself had no effect upon growth. Sulfanilamide at a level of 10 mg per 100 ml prevented growth entirely over the period of observation. In the presence of 25 mg % of sulfanilamide the addition of .01 mg % p-amino benzoic acid (a molecular ratio of 2,000:1) permits the reaction to proceed at about ½ the maximum rate. This does not take into account p-amino benzoic acid which may have been synthesized by the organism.

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[5] Elvehjem *et al.*, *Respiratory Enzymes*, Minneapolis, 1939.

[6] MacLeod, C. M., *J. Exp. Med.*, 1940, **72**, 307.

The logarithms of the turbidity readings were plotted against time and the slope of the resulting straight lines was used as a measure of the growth rate. The lines were fitted to the experimental points by the "method of the least squares" to obtain the best estimate of the slope (b) and the error (s^2). It will be observed that the errors are very small, indicating an excellent fit of the data to the lines, and that the series of errors is homogeneous. The velocity constant *k* which is the best estimate of the velocity of the reaction (v), is 2.303 times the slope of the line since common logarithms were used in the plot.

Having determined the velocity of growth in the presence of various concentrations of substrate and inhibitor the reciprocal of

TABLE I.
Effect of Sulfanilamide and Para-amino Benzoic Acid on Growth Rate of *E. coli.*

Conc. mg%		Turbidity (arbitrary units*)								
Sulfanilamide	P.A.B.	3	4	5	6	7 hrs	b†	k	1/k	s^2‡
25	0	.4	.4	.4	.4	.3	—.025	0.00	∞	.0021
25	.01	1.5	2.2	2.6	3.6	5.7	.137	.316	3.16	.0014
25	.03	1.6	2.6	3.8	6.8	11.4	.212	.489	2.04	.0010
25	.05	1.9	3.1	5.5	9.6	16.7	.238	.549	1.82	.0003
25	.1	2.0	3.5	6.1	11.5	20.0	.252	.581	1.72	.0001
25	.3	2.2	4.3	9.0	15.5	27.0	.273	.629	1.59	.0009
10	0	.5	.5	.4	.5	.5	.000	.000	∞	.0025
10	.005	1.7	2.8	4.4	6.9	11.0	.201	.463	2.16	.0001
10	.01	2.0	3.2	6.2	9.6	17.0	.234	.540	1.85	.0006
10	.03	2.3	4.1	7.6	13.4	24.0	.255	.587	1.70	.0000
10	.05	2.3	4.4	8.9	15.0	27.0	.267	.615	1.63	.0006
10	.1	2.3	5.1	10.0	16.9	30.0	.275	.634	1.58	.0018
0	.005	2.5	5.0	10.0	18.0	32.0	.277	.638	1.57	.0006
0	.01	2.3	5.4	10.6	17.6	33.0	.283	.652	1.53	.0023
0	.03	2.4	5.3	10.0	17.8	33.0	.280	.645	1.55	.0010
0	.05	2.4	5.0	10.0	18.1	32.0	.281	.648	1.54	.0008
0	.1	2.4	5.3	10.0	17.8	33.0	.280	.645	1.55	.0010
0	.3	2.4	5.3	10.0	17.6	33.2	.280	.645	1.55	.0010
0	0	2.4	5.2	10.0	18.9	33.0	.283	.652	1.53	.0009

*1 unit = 8 million *E. coli.*
†Slope of plot of log. turbidity vs. time.
‡Error in plot of log. turbidity vs. time.

the velocity was plotted against the reciprocal of the substrate concentration. The results shown in Fig. 1 substantiate the theory of competitive inhibition since (1) the plots give straight lines as postulated, (2) the presence of the inhibitor has no effect on the intercept which remains as the reciprocal of the maximum velocity ($1/V = 1.55 \pm 0.02$) regardless of inhibitor concentration, and (3) the concentration of inhibitor determines the slope of the lines.

The statistical presentation of these data appears in Table II. The intercepts ($1/V$) of 1.54, 1.57 and 1.53 do not differ significantly. The experimental data fit very well to the straight

TABLE II.
Summary of Statistical Analysis.

Sulfanilamide concentration	N	Equation of line	variance (s^2)	t	$t^*_{0.01}$
mg%					
0	6	$1/k = .0001\ 1/cP.A.B. + 1.54$	.0002	17.6	3.50
10	5	$1/k = .0029\ 1/cP.A.B. + 1.57$	.0006	12.3	3.70
25	5	$1/k = .0162\ 1/cP.A.B. + 1.53$	.0013		

cP.A.B. = concentration p-amino benzoic acid.

*From Fischer's t table. When t exceeds $t_{0.01}$ the probability is less than 1 in 100 that the difference in slopes is due to chance alone.

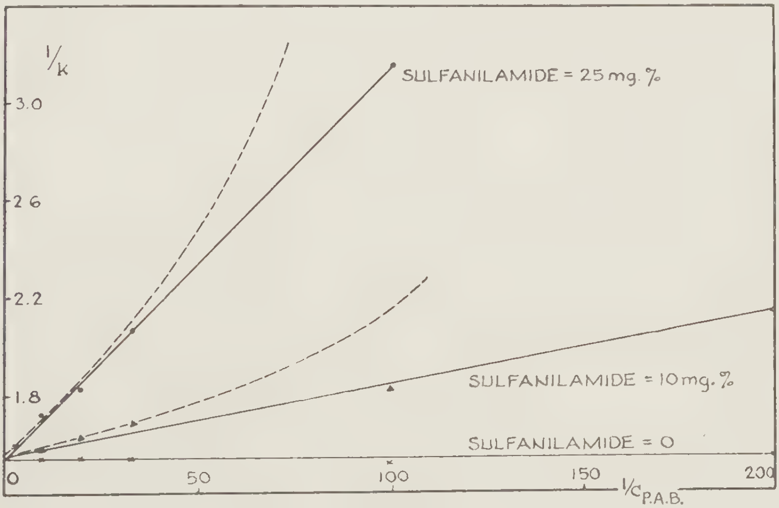

FIG. 1.

Test for Type of Inhibition by Sulfanilamide.

Ordinate = 1/velocity constant. Abscissa = 1/concentration p-amino benzoic acid. Solid lines are fitted to actual experimental points. Dotted lines indicate the plot if the organisms contained 0.005 mg% p-amino benzoic acid in addition to that which was added.

lines as is indicated by the small variance (s^2). Application of the *t* test[7] reveals that the differences in slopes are far in excess of the requirements for significance.

The fact that maximum growth velocity occurs with *E. coli* in the absence of any added p-amino benzoic acid if sulfanilamide is also absent does not refute the thesis of competitive inhibition. P-amino benzoic acid may be present as an impurity in the medium or more likely, it may be synthesized by the organism as required. That such amounts are very small is demonstrated by the plot of the broken lines in Fig. 1 which show how the graph would appear if 0.005 mg % of p-amino benzoic acid were present, either as impurity or as a product of bacterial synthesis, in addition to the added amount. Obviously such is not the case. However, since Rubbo and Gillespie[3] showed that as little as .00002 micrograms permitted growth of *Cl. acetobutylicum,* the amount required for growth could be present without noticeably distorting the plot. Admittedly, alternative explanations are available. Any one of a number of proposed mechanisms for sulfonamide action may obtain but it is now possible to reject all but those in which competitive inhibition is characteristic. The data presented here clearly demonstrate that sulfanilamide and p-amino benzoic acid behave as if they were competing for the same receptor site in the organism. The tremendous disproportion between the molecular concentrations merely indicates the greater affinity of the site for the p-amino benzoic acid.

Summary. Growth rates of *E. coli* were measured in the presence of sulfanilamide and p-amino benzoic acid. Mathematical analyses of the data indicate that the sulfanilamide inhibition is of the competitive type.

13243 P

Pathogenesis of Erythroblastosis Fetalis: Absence of the Rh Factor from Saliva.

PHILIP LEVINE AND E. M. KATZIN.

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The pathogenesis of erythroblastosis fetalis has been ascribed to the isoimmunization of the mother by the Rh, or, more rarely, by other factors in the blood of the fetus.[1, 2] According to this concept maternal agglutinins produced in response to this stimulus after passing the placenta, act on the susceptible blood of the fetus, and thus produce the various syndromes known as erythroblastosis fetalis. This theory does not differ in principle from the older concept of "heterospecific pregnancy"[3] based on the 4 blood groups. Thus, if the property A or B in the fetus is not present in the mother, the maternal agglutinin by its action on the fetal blood was thought to induce icturus gravis, (one of the manifestations of erythroblastosis fetalis).[4] Recently it was shown by Jonsson,[5] and confirmed by Levine,[6] that in the example cited the mother's normal agglutinins may be specifically increased in titer as a result of isoimmunization.

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[7] Fisher, R. A., *Design of Experiments,* London, 1937.

Nevertheless, the theory of heterospecific pregnancy was abandoned because of lack of evidence.[7] Furthermore, the demonstration that the factors A and B are present, also, in tissue cells and body fluids indicate that maternal isoantibodies are specifically inactivated after passing the placenta. However, this wide distribution of the A and B blood factors applies only to about 80% of individuals (secretors) and in the remainder (non-secretors) the A and B substances are presumably limited to red blood cells.[8, 9, 10]

These facts suggested experiments to determine whether the Rh factor is also present in tissue cells or in body fluids. The tests were carried out with saliva because of its high concentration of the A and B substances.

For control, the tests, which were limited to saliva from individuals of groups A and B, were carried out simultaneously with isoantibodies anti-A and anti-B of a group O serum and an anti-Rh serum. The latter serum was obtained from a woman (Group A) who had recently delivered an infant with erythroblastosis fetalis.

Equal volumes of saliva, clarified by prolonged centrifuging and suitable dilutions of serum were incubated at 37°C for one hour and overnight in the ice-box. The tests with the anti-Rh serum — saliva

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[1] Levine, P., Katzin, E. M., and Burnham, L., *J. A. M. A.*, 1941, **116**, 825.

[2] Levine, P., Vogel, P., Katzin, E. M., and Burnham, L., *Science*, 1941, **94**, 371.

[3] Hirszfeld, L., *Konstitutionsserolgie und Blutgruppenforschung,* Julius Springer, Berlin, 1928.

[4] Ottenberg, R., *J. A. M. A.*, 1923, **81**, 295.

[5] Jonsson, B., *Acta Path. et Miscrobiol. Scand.*, 1936, **13**, 424.

[6] Levine, P., unpublished data.

[7] Koller, S., *Z. f. Rassenphysiol.*, 1930, **3**, 121.

[8] Putkonen, T., *Acta Med. Fenn. "Duodecim,"* 1930, **14**, 107.

[9] Schiff, F., and Sasaki, H., *Klin. Woch.*, 1932, **11**, 1426.

[10] Friedenreich, V., and Hartmann, *f. Immunitätsf.*, 1938, **92**, 141.

TABLE I.
Specific Inhibition of Anti-A, Anti-B, and Anti-Rh by Saliva.

		I Serum Group O Incubated with saliva Tested with blood suspension of group		II Serum M.F. Anti-Rh Incubated with saliva	
Saliva donor	Group	A	B	Tested with blood O Rh+	Rh reaction
1.	A	0	++	+±	+
2.	A	0	++	+±	+
3.	A	0	++	+±	0
4.	A	0	++	++	+
5.	A*	++	++	++	+
6.	B	++	0	++	+
7.	B	++	0	++	+
8.	B	+±	0	++	+
9.	B	++	0	+±	0
10.	B*	++	++	++	+

Before adding blood, the test mixture consisted of 0.2 cc saliva and 0.2 cc serum dilution. The final dilution of the group O serum was 1:16; that of the anti-Rh serum was 1:8. Readings in I were made after the test stood 2 hours at room temperature; in II the readings were made after the test stood for 1 hour at 37°C.

*Non-secretor.

mixtures were made by adding 3 drops of a washed blood suspension of group O, Rh +. The mixtures with the group O serum were divided into 2 series, to one of which was added a group A blood-cell suspension and to the other, group B blood.

Representative experiments with the saliva of 5 selected individuals of group A and 5 of group B are recorded in Table I.

The results indicate that this technic readily reveals the presence of group specific substances A and B in the saliva. Under these experimental conditions, however, the saliva of Rh+ and Rh— individuals cannot be differentiated. These findings indicate that the Rh blood factor is not present in saliva.[†] Saliva samples of 63 individuals were tested (41 of Group A, 9 of whom were non-secretors, and 22 of Group B, only one of whom was a non-secretor). Of the total, 10 were found to be Rh—, 5 in each of Group A and B.

Sperm cells and seminal fluid of 3 Rh+ individuals of Group O were tested for their capacity to absorb or inhibit the action of the anti-Rh agglutinin. These results indicated that the Rh factor was not present in the material examined.

Studies on the distribution of the A and B substances in tissue cells and body fluids revealed that their presence in saliva is an index of their wide distribution throughout the body.[8, 10, 11] Accordingly, there is some justification to assume that the Rh factor may be limited to red blood cells only. However, a comprehensive study of various organs and body fluids is desirable.

[†] A similar observation based on several specimens of saliva was reported by Wiener and Forer (PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 215).

[11] Sasaki, H., *f. Immunitätsf.*, 1932, **77**, 101.

The absence of the Rh factor from the tissue cells and body fluids might have been anticipated on the basis of the evidence indicating the importance of the Rh factor in the pathogenesis of erythroblastosis fetalis.[12]

13244 P

Succinyl Sulfathiazole, a New Bacteriostatic Agent Locally Active in the Gastrointestinal Tract.

EDGAR J. POTH AND F. LOUIS KNOTTS. (Introduced by W. M. Firor.)

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Much interest has been aroused during the past year over the prospect of developing therapeutic agents that have antibacterial activity in the bowel.[1, 2, 3] A study covering 20 sulfonamides has unearthed a compound of considerable promise.

Succinyl sulfathiazole.* $COOH . CH_2 . CH_2 . CO . NH . C_6H_4 . SO_2 . NH . C_3H_2NS$, has been shown to have little toxicity when administered to dogs orally at 4-hour intervals day and night. A dosage of one gram per kilo daily given in 6 equal doses results in a fecal drug content of from 5 to 10%. Under this regimen approximately 5% of the ingested drug is excreted in the urine, and the concentration of the drug in the blood will average 1.5 mg % of sulfathiazole and 2.0 mg % of succinyl sulfathiazole. The sulfathiazole is formed by the hydrolysis of a small portion of the conjugated compound by the animal tissues and by the bacteria in the bowel. Crystals of the drug do not appear in the urine.

The antibacterial action of succinyl sulfathiazole in the bowel as measured by the effect on the coliform organisms is presented graph-

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[12] Levine, P., Burnham, L., Katzin, E. M., and Vogel, P., *Am. J. Obst. and Gyn.*, in press.

[1] Marshall, E. K., Jr., Bratton, A. C., White, H. J., and Litchfield, J. T., Jr., *Bull. J. H. H.*, 1940, **67**, 163.

[2] Firor, W. M., and Jonas, A. F., *Ann. Surg.*, 1941, **114**, 19.

[3] Firor, W. M., and Poth, E. J., *Ann. Surg.*, in press.

*This compound was furnished by Sharp and Dohme of Philadelphia, Pa.

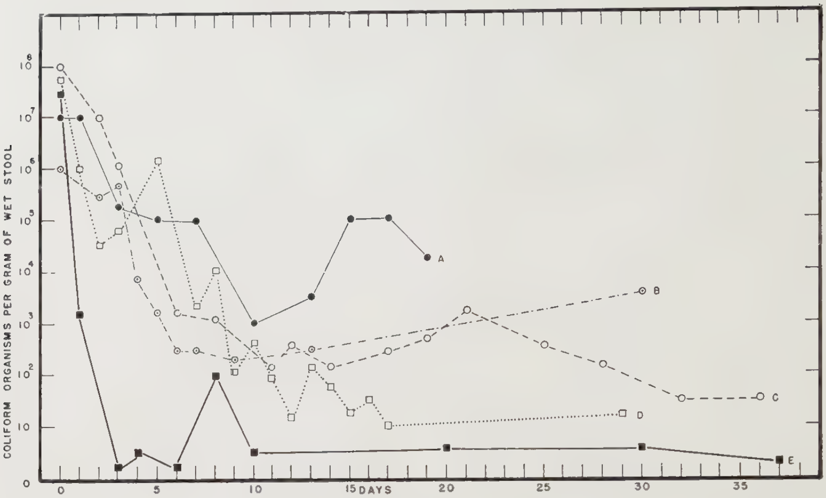

Fig. 1.

The graphic representation of the effect of various dosages of succinyl sulfathiazole on the coliform organisms in the gastrointestinal tract of the dog. Curve *A* diagrams the results following the oral administration of 0.05 g per kilo per day given in 6 equal doses at 4-hour intervals; *B* likewise for 0.1 g per kilo; *C* for 0.25 g per kilo; *D* for 0.50 g per kilo; and, *E* for 1.0 g per kilo.

ically. The drug given in therapeutic doses profoundly alters the feces. They become semifluid and practically odorless.

No toxic manifestations have been observed. The animals gain weight and show no gross or microscopic evidence of tissue damage after receiving one gram per kilo of the compound daily in 6 doses for 5 weeks. During this entire period the number of *B. coli* per gram of wet stool averaged less than 100 organisms as compared to an average normal flora of 10,000,000.

Succinyl sulfathiazole has been administered to 40 human beings with various organic lesions and acute intestinal infections without the production of toxic reactions. The stools become semifluid and relatively odorless. The coliform organisms are reduced to such low values that *B. coli* frequently cannot be demonstrated by growth on desoxycholate plates.

The activity of this compound is limited largely to its local effect on the contents of the gastrointestinal tract.

13245

Rôle of the Auricles in Ventricular Filling of the Tortoise Heart.

Stephen W. Gray. (Introduced by F. R. Steggerda.)

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Studies of the tortoise heart *in situ* were made with a view to determining the extent to which ventricular filling is due to auricular systole. From observation it was obvious that such an effect existed, although it was difficult to show by means of a kymograph and lever system.

The cinematographic method, originally employed by Takeuchi[1] and Strughold[2] and lately Gray and Steggerda,[3] was used to record size changes and resulted in a clear picture, free from artifacts of inertia and overthrow common to mechanical systems. Pictures were taken at 8 per second and various diameters of the ventricle and auricle in each picture were measured under a binocular so that any change in diameter could be plotted graphically and used for further study.

In a typical curve from a slowly beating heart there is shown a definite increase in ventricular size just prior to systole. In all cases this coincides with the contraction of the auricles as indicated by the curve of auricular diameter. To examine the possibility that this auricular effect might be exaggerated by the absence of the restraining influence of the pericardium, experiments were done in which the beating heart was photographed through the pericardium. The measurements showed no difference between these hearts and those in which the pericardium had previously been removed. To further test the validity of this auricular effect on ventricular filling, the auricles were ligated in such a manner as to allow a clear passage for the blood from the sinus venosus to the ventricle, but at the same time preventing blood from entering the fundus of the auricles. By this operation, the mechanical effect of the auricular systole was abolished without impeding the path of either the blood or the excitation wave. The presystolic dilatation due to the auricular systole was completely absent, but the remaining part of the ventricular beat was relatively unchanged.

When the relation of the auricular contribution to ventricular

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[1] Takeuchi, K., *J. Physiol.*, 1925, **60**, 208.

[2] Strughold, H., *Am. J. Physiol.*, 1930, **94**, 641.

[3] Gray, S. W., and Steggerda, F. R., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 269.

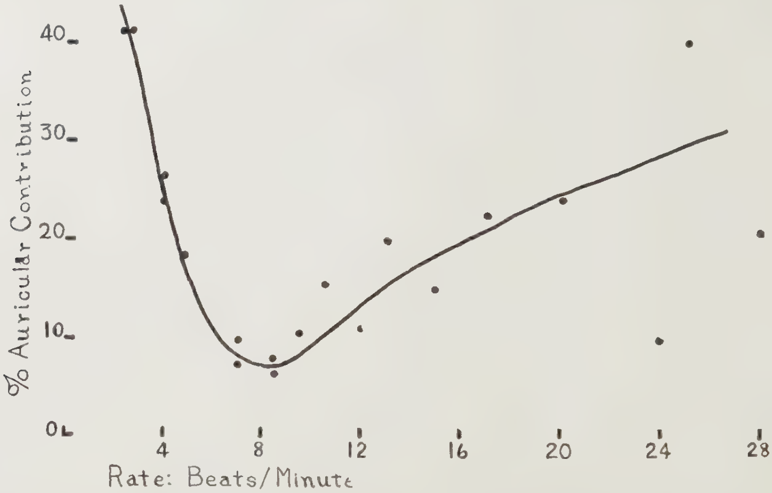

FIG. 1.

Relation between the auricular contribution to the diameter of the ventricle and the rate of the heart beat.

size was compared in hearts beating at different rates, the effect of the auricles was found to be markedly different. From Fig. 1, it is shown that with a rate of about 8 beats per minute, the auricular contribution amounts to from 7 to 10% of the ventricular diameter. However, as the rate varies in either direction from this figure, the action on the ventricular size is increased so that at 3 beats per minute, as well as at 25 per minute, the auricular contribution is about 20% to 40% of the total ventricular diameter. The figures represent linear change and not actual volume changes which would be rather greater as the increase takes place in 3 dimensions. As to an exact explanation for these differences, little can be said at the present time, but it would seem that there is an optimum rate at which the ventricle is doing its greatest amount of work and the auricles their least. The rate of 8 per minute corresponds roughly with the environmental temperature of 20 to 25°C.

13246

Composition of the Milk of the Monkey (*M. mulatta*).

G. van Wagenen, H. E. Himwich and H. R. Catchpole.

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Apart from the observations of Schumacher[1] on the Orang-utan, we have found no data in the literature relative to the composition of milk in the infrahuman primates. We present here a summary of some analyses made by one of us (H. E. H.) on milk samples taken from 4 monkeys (I-IV) during 1936-37, and from another group of 5 monkeys (V-IX) during 1938-39.

Methods. The babies were removed from their mothers 4 hours prior to collection of the sample. Milk was expressed by hand, particular care being taken to avoid traumatic changes in the nipples. Usually 4 to 8 cc of milk could be obtained. Samples were taken after lactation had become well established. In monkey V, specimens were obtained between the 35th and 50th day; in monkey VII, between the 61st and 103rd day, and in monkey VIII, between the 44th and 75th day. During these intervals, at least, no systematic changes were observed in the percentages of the principal milk constituents, or in the calorific values ascribable to them. Results on the two groups of animals are presented in Tables I and II.

TABLE I.
Composition of Monkey Milk (H.E.H. data).

Monkey No.	Protein[1] Average %	Protein[1] % Calories	Fat[2] Average %	Fat[2] % Calories	Carbohydrate[3] Average %	Carbohydrate[3] % Calories
I	(7)* 3.56	18	(11) 4.58	53	(5) 5.62	29
II	(6) 2.64	10	(16) 7.65	66	(7) 6.14	24
III	(6) 1.51	10	(6) 3.44	50	(4) 6.34	40
IV	(5) 1.77	12	(4) 2.51	39	(1) 7.10	49

[1] Micro Kjeldahl method. [2] Man and Gildea method. [3] Hydrolyzed with HCl: Hagedorn and Jensen method.

*Figures in parentheses refer to the number of samples analyzed.

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* The monkey colony which is maintained for the study of reproductive physiology in the laboratory of the Department of Obstetrics and Gynecology at New Haven has been supported by grants from the Fluid Research Fund of the Yale University School of Medicine and by the John and Mary R. Markle Foundation.

[1] Schumacher, H. M., *Z. Kinderheilk.*, 1934, **56**, 415.

TABLE II.
Composition of Monkey Milk (H.R.C. data).

Monkey No.	Protein†		Fat‡		Carbohydrate§		Specific gravity	Ash %
	Average %	% Calories	Average %	% Calories	Average %	% Calories		
V	(10)* 2.60	14.5	(10) 4.50	56.0	(10) 5.45	29.5	(5) 1.010	(5) .27
VI	(1) 1.45	15.0	(1) 2.45	56.5	(1) 2.80	28.5		
VII	(3) 1.77	11.8	(3) 3.27	48.5	(3) 6.20	39.8	(3) 1.019	(3) .26
VIII	(3) 2.05	14.9	(3) 2.40	39.6	(3) 6.31	45.5	(3) 1.024	(3) .25
IX	(1) 1.45	8.1	(1) 4.34	54.0	(1) 7.01	38.0		

†Semi-micro-Kjeldahl, Hitchcock and Belden[2] (nitrogen:protein = 1:6.37). Average of duplicates. ‡Meigs' method. §Benedict's method applied to cleared filtrate. Duplicates.
*Figures in parentheses refer to the number of samples analyzed.

Discussion. Protein. In the first group of animals, monkey I showed a high milk protein value, which represented 18% of the total calories of the milk. This does not appear to be a typical finding. All other animals in this group showed caloric percentages for protein of 10-12, which approximates closer to the human figure (9%) than to that typical of the cow (22%). Further, the second group of results definitely confirmed the impression that the protein of monkey milk both in absolute (1.45-2.60%) and in caloric (8.1-15.0%) percentages tends rather toward the values recorded for man and the orang.

Carbohydrate. The sugar content of monkey milk is some 25% higher than that of cow's milk and approximates closely that of man and the orang. In caloric percentage attributable to carbohydrate, monkey milk lies intermediate between that of man and cow.

Ash. Monkey milk resembles human in its low ash content (0.26%) as compared with that of the cow (0.6-0.7%).

Average values for the group of 9 animals give: protein 2.1%, carbohydrate 5.9%, fat 3.9%. The corresponding caloric values are 12.6%, 34.7%, 52.8%. As an expedient for feeding valuable monkey babies unable to suckle their mothers for any reason, cow's milk must be modified in the same general direction as for human infant feeding. Practically, 2 oz water and 4.1 g sucrose (level teaspoon) are added to 4 oz cow's milk. Protein and ash are thus reduced in amount and calories ascribable to carbohydrate are increased.

Summary. 1. Monkey's milk tends toward that of the human in composition and differs from cow's milk in having a lower percentage of protein and ash and a higher percentage of milk sugar. 2. A modification of cow's milk for infant monkey *(M. mulatta)* feeding is described.

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[2] Hitchcock, D. I., and Belden, R. C., *Indust. Engr. Chem.*, 1933, **5**, 402.

13247

Estrogen Intranasally in Experimental Poliomyelitis.*

EDWIN W. SCHULTZ.
From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

Aycock[1] has reported that castrated immature female monkeys given estrogenic hormone intramuscularly showed a higher level of resistance to intranasal inoculation with poliomyelitis virus than untreated castrates. His studies apparently were prompted in part by endocrinological observations showing that nasal mucosal changes are associated with the changes induced in the vaginal epithelium by estrogenic hormone under experimental conditions. Prominent among the observed genital responses is a thinning of the vaginal epithelium following castration and its restoration to normal with the administration of estrogenic hormone. However, so far as we are aware the nasal changes reported deal only with vascular responses in the region of the inferior and middle turbinates.[2] Possible changes in the olfactory portion of the nasal mucosa apparently have thus far not been considered.

Inasmuch as it has been established that in monkeys inoculated with poliomyelitis virus by the intranasal route the portal of entry is through the olfactory portion of the nasal mucosa, the results obtained by Aycock suggested the desirability of an experiment in which estrogen would be brought into direct contact with the olfactory mucosa for a number of days, immediately following which some of the animals would be tested for resistance to virus by the intranasal route and the olfactory mucosae of others would be examined histologically for possible anatomic responses.

Fifteen uncastrated female *Macacus rhesus* monkeys were given theelin in oil (10,000 International Units per cc solution†) by intranasal instillation, daily, as indicated in Table I. The instillations were made while the animals were under deep ether anesthesia and held in the fully inverted position in a special animal holder. They were made with a Luer syringe, to which was attached a 1-inch

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* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

[1] Aycock, W. L., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 573; *Am. J. Publ. Health*, 1937, **27**, 575; *Endocrinology*, 1940, **27**, 49.

[2] Mortimer, H., Wright, R. P., and Collip, J. B., *Canad. Med. Assn. J.*, 1936, **35**, 503.

† Supplied through the courtesy of Dr. Oliver Kamm, Parke Davis and Co.

needle with a blunt point. About 0.25 cc of the solution was introduced drop by drop, deep into each nasal passage. During the instillation the mouth of the animal was held open and the tongue pulled forward to reduce nasal breathing and discharge of fluid before it could gravitate to the olfactory area. The condition of the sexual skin was recorded at the time of the first instillation and each day thereafter during the course of the treatments. Animals which showed no appreciable redness and swelling of the sexual skin were recorded as —; those which showed maximal redness and swelling were recorded as ++++.

The animals used in the experiment fall into 3 groups: (1) a group of 8 which received instillations of theelin daily over periods ranging from 1 to 8 days. One animal out of the group was sacrificed each day to determine the onset of any histological change which might occur in the olfactory mucosa, (2) a group of 7 animals which received theelin once daily for 8 days, and which were then tested for resistance to intranasal infection. The resistance test consisted of 2 intranasal instillations of a 10% suspension of virus (MV strain) on successive days. These virus instillations were made with a bulb urethral syringe, while the animals were under ether anesthesia and held in the fully inverted position. The first instillation was made 1 day, the second 2 days following the 8th instillation of theelin, (3) a group of control animals, consisting of 6 females of about the same age and weight as the test animals. None of the group showed appreciable reddening or swelling of the sexual skin.

All of the animals, including the controls, were sacrificed by exsanguination and the tissues fixed by the perfusion method. In most of the animals Bouin's solution was used; in several Bodian's formula No. 2[3] was substituted. The olfactory mucosa from both sides was removed, along with the corresponding olfactory nerve filia and bulbs. These parts were removed as one connected mass. After removal of the tissue, the parts were restored as nearly as possible to their normal relationship and put in fixing solution for 18-24 hours. The tissue was then dehydrated in graded alcohols, beginning with 35% solution and finally embedded in paraffin. It was sectioned through the coronal plane, so that each section presented 4 mucosal surfaces, the olfactory nerve filia and the olfactory bulbs. Part of the sections were stained with H and E, part of them by Bodian's method for neurofibrils.[3]

The results of this experiment are summarized in Table I.

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[3] Bodian, D., *Anat. Record*, 1936, **65**, 89; 1937, **69**, 153.

TABLE I.

Group 1.

Animals given 1 to 8 instillations of theelin, daily. One animal was sacrificed each day for histological observations.

			Sexual skin		
Animal No.	Wt, lb	No. daily instillations of theelin	At beginning of treatment	End of treatment	Olfactory mucosa
E 602	6¼	1	—	—	No distinctive epithelial changes recognized
E 603	5¼	2	—	—	
E 604	4	3	—	—	
E 605	6¼	4	+	+++	
E 606	7¼	5	++++	++++	
E 607	6½	6	++	++++	
E 608	6	7	+	+++	
E 609	6	8	+	++++	

Group 2.

A group of animals given 8 instillations of theelin, daily, followed 1 and 2 days later by intranasal instillations of 10% suspension of virus.

		Sexual skin					
Animal No.	Wt, lb	Beginning of treatment	End of treatment	Onset of poliomyelitis, days after 1st inoculation with virus	Time sacrificed, days after 1st inoculation with virus	State of sexual skin at time sacrificed	Olfactory mucosa
E 610	5¾	+	++++	8	12	±	No distinctive epithelial changes recognized
E 611	5½	+	++++	10	12	±	
E 612	6⅞	+++	++++	10	12	+	
E 613	5	+	++++	8	9	±	
E 614	6½	+	++++	8	12	—	
E 615	5¾	+	++++	9	10	±	
E 616	4⅞	+	+++	10	12	+	

Group 3.

Control group. Two instillations of virus as in group 2.

Monkey No.	Wt, lb	Sexual skin	Onset of poliomyelitis, days after 1st inoculation with virus	Olfactory mucosa
E 618	5⅛	—	8	These and other "normal" mucosæ served as basis for comparison
E 619	6	—	10	
E 620	5	—	8	
E 621	6	—	10	
E 624	5¼	—	10	
E 625	6¼	—	7	

It is apparent that under the conditions of this experiment theelin-treated animals showed about the same susceptibility to infection by the intranasal route as the untreated control animals. Whether a difference might have been demonstrated had the theelin treatments been continued over a longer period of time, or a lower concentration of virus had been employed in testing the resistance to infection, is hard to say. There is, however, nothing in these results to suggest that this might have been the case.

Other than a slight degree of leucocytic infiltration the olfactory mucosae from theelin-treated animals showed no structural features which failed to fall in with variations met with in "normal" olfactory mucosae. While individual mucosae in Group 1 showed epithelia which were above average in thickness, others obtained from animals treated about as long with theelin, showed epithelia which were distinctly below average. There was no evidence of unusual proliferative activity. In 2 mucosae there was evidence of some antemortem detachment of epithelial cells. Whether there is normally some detachment of the olfactory epithelial cells with relation to the menstrual cycle remains a question. In a later paper, in which we shall deal with variations in the olfactory mucosae of normal monkeys, reference will be made to observations suggesting that the olfactory epithelium is not infrequently called upon to repair losses due to natural causes.

The group (Group 2) tested for resistance to poliomyelitis virus showed about the same degree of round cell infiltration along the olfactory pathway[4] as was present in the untreated control group.

What impressed us most in the results of this experiment was the striking response of the sexual skin to the intranasal instillation of estrogen, confirming an already recognized fact that physiologically active drugs are readily absorbed from the nasal mucosa.

13248

Failure of Sulfanilamide in Treatment of Experimental Vaccinia Rabbits.

JOHN A. KOLMER AND HERMAN BROWN. (With the technical assistance of Anna M. Rule.)

From the Research Institute of Cutaneous Medicine, Philadelphia, Pa.

McCammon[1] reported that the early administration of sulfanilamide to 4 cases of benign smallpox appeared to have a beneficial effect upon the disease. In the same year Pierna[2] reported on the treatment of 7 cases with the compound and since vesiculo-pustular evolution of the lesions, desiccation and peeling were accomplished in less than half the time observed in untreated controls, thought that sulfanilamide may attenuate the virus of smallpox and prove specific in the treatment of the disease.

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[4] Sabin, A. B., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 300; *J. A. M. A.*, 1937, **108**, 21.

[1] McCammon, W. O., *J. A. M. A.*, 1939, **112**, 1936.

Since cowpox virus is biologically closely related to smallpox virus, we thought it advisable to study the effects of sulfanilamide upon experimental vaccinia of rabbits in order to determine more definitely whether or not the compound had any appreciable clinical effects upon the virus and the lesions produced by cutaneous inoculation. To the best of our knowledge no previous investigations have been reported and we may state at the outset that sulfanilamide has proven ineffective. It may be, however, that the compound is helpful in the treatment of smallpox and likewise in generalized vaccinia of human beings by reducing the degree of secondary infection of vesicles with staphylococci and streptococci from the skin, but since sulfanilamide has had no apparent effect upon experimental vaccinia of rabbits, we believe it quite unlikely to be effective in the treatment of smallpox insofar as infection with the virus is concerned.

Twenty-four rabbits, weighing from 1950 to 2300 g, were employed. The abdomen of each was shaved, the skin scarified and inoculated with seed virus* in the usual manner. Papular lesions were apparent within 48 hours after inoculation with vesiculation on or about the fifth day. Regression began on the seventh to eighth days with healing on the twelfth to fourteenth days. None of the rabbits succumbed.

At intervals of 24, 48, 72, 96 hours and 5 days after inoculation treatment was started, 4 rabbits being used at each interval with 4 untreated controls. Sulfanilamide was administered orally in dose of 0.1 g per kilo, 2 doses being given daily (10 A.M. and 4 P.M.) amounting to 0.2 g per kilo per day and equivalent to 12 g daily per 60 kilo, or approximately 132 pounds of body weight. Each animal received from 10 to 14 doses.

That the compound was absorbed is indicated by the fact that the blood concentrations of free sulfanilamide 4 hours after the first dose varied from 0.7 to 1.8 mg per 100 cc; about 1.5 mg after the third dose and 1.0 to 2.2 mg after the fifth dose. Two hours after the fifth dose the concentration varied from 2.0 to 3.2 mg; 1.48 to 2.16 after the seventh dose; 2.16 to 2.4 mg after the ninth dose;

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[2] Pierna, S., *Rev. Espãn. de Med. y. Cir. de Guerra, Valladolid*, 1939, **3**, 279.

* Kindly furnished by the Sharp & Dohme Laboratories, Glenolden, Pa.

3.0 to 3.6 mg after the eleventh dose and 2.96 to 3.0 mg after the thirteenth dose.

The results were entirely negative as the compound in the dosage administered was without apparent effect upon the evolution and course of the vaccinal lesions.

Conclusions. 1. Sulfanilamide by oral administration in dosage of 0.1 g per kilo of weight, twice daily for 10 to 14 doses, had no appreciable effect upon the evolution and clinical course of experimental vaccinia of rabbits. 2. Under the circumstances it is unlikely that sulfanilamide has any effect upon the virus of smallpox, although its administration may be helpful in the treatment of smallpox and generalized vaccinia by reducing secondary staphylococcus or streptococcus infections of the vesicles.

13249

Cultivation of Influenza A Virus in Roller Tubes.

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The prolonged cultivation of vaccinia in roller tube cultures of chick embryonic tissue has been reported.[1] After an initial rise, individual cultures were observed to maintain a high titer of virus for as long as nine weeks. The present communication presents the results obtained in the cultivation of influenza A virus by means of the same method.

The Melbourne strain of influenza A virus which had been passed exclusively in eggs or in chick embryo tissue culture for more than 300 transfers was used. Introduced intranasally 0.05 cc of a suspension of infected embryo diluted 10^{-4} or 10^{-5} in infusion broth killed all mice. Cultures were inaugurated by mixing a suspension of virus with finely minced, 10-day chick embryo (head removed). About 0.6 cc of infected tissue was distributed over the layer of chicken plasma on the inner surface of the lower half of a roller tube. Nutrient fluid consisted of 1.6 cc of so-called 7-2-1 mixture (Simms' solution 7 parts, chick embryo extract 2 parts, chicken serum 1 part). This fluid was changed daily when 20 cc of sterile

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[1] Feller, A. E., Enders, J. F., and Weller, T. H., *J. Exp. Med.*, 1940, **72**, 367.

TABLE I.
Titration in Mice of Virus in Tissue and Fluid Components of Roller Tube Cultures After Various Intervals of Incubation at 37°C.

	Age of culture in days							
	5		10		20		30	
Dilution	T	F	T	F	T	F	T	F
10^{0}	D	D	D	D	D	D	D	D
10^{-2}	D	+	D	+	D	+	D	+
10^{-3}	nd	+	nd	+	nd	+	nd	+
10^{-4}	+	nd	+	nd	+	nd	+	nd

T = tissue.
F = fluid.
D = 50% or more of mice died within 10 days with typical lung lesions.
+ = mice survived 10 days but had lung lesions at autopsy.
nd = not done.

air were blown into the tube. At intervals of 5 to 30 days the tissues and fluids from the cultures were tested separately for their content of virus. The tissue was ground in 1.6 cc of broth and the resulting suspension designated as a 10^{0} dilution. Serial dilutions of this tissue suspension and of the nutrient fluid were then administered intranasally to mice. The titer of virus was taken as that dilution which in a volume of 0.05 cc killed 50% or more of the mice within 10 days after intranasal inoculation. The results of these titrations are given in Table I.

The respective titers of tissue and fluid were fairly constant for at least 30 days. Furthermore, the titer or persistence of the virus did not vary with the amount of virus in the inoculum.

In other experiments the composition of the nutrient fluid was altered. The effect of such alterations on the increase and maintenance of the virus was then determined. Tyrode solution instead of the 7-2-1 mixture was first used. The results were entirely comparable on the 5th day of cultivation to those obtained at this time with the more complex fluid. At 10 days, however, there was less virus present and much less at 15 days. After 20 days only enough virus remained in both undiluted fluid and tissue suspension to produce small lesions in mice. No virus was detected at 25 days. When the Tyrode solution was not changed and the culture left stoppered until tested (tissue plus fluid tested together), as much virus was present at 5 days as if the fluid had been changed daily; at 10 and 15 days the amount of virus had decreased and at 20 days was barely detectable; by 30 days it had disappeared. These findings are in agreement with those reported by others[2] on the survival of influenza virus in a closed culture.

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[2] Magill, T. P., and Francis, T., Jr., *J. Exp. Med.*, 1936, **63**, 803.

When a 0.9% solution of sodium chloride was substituted for the 7-2-1 mixture, the results were as follows: at 3 days both tissue and fluid contained as much virus as the cultures in 7-2-1; at 6 days there was slightly less virus in the tissue and much less in the fluid; at 9 days virus was barely detected in the tissue but was not found in the fluid and none was demonstrated in either component at 13 days. It is apparent from these observations that for relatively short term experiments the fluid component of the roller tube culture may be composed of very simple constituents—a fact of practical value should the method be employed in metabolic studies on tissues infected with virus.

An attempt was made to determine which tissues of the chick embryo in culture permitted the multiplication of virus. Previously it was shown[3] that egg-adapted strains induced rapidly fatal, generalized infections of the embryo *in vivo* in contrast to certain strains partially adapted to the egg which did not involve the embryo after inoculation on the chorio-allantoic membrane. Inoculation of the latter into the amniotic sac, however, resulted in lung lesions and a high titer of virus in the lung and amniotic fluid. When virus was inoculated between the yolk sac and chorio-allantois,[4] it was recovered in high titer from the yolk sac and in even higher titer from the chorio-allantois and amnion and from the amniotic fluid.[5, 6]

The capacity of various organs from 14-day chick embryos to

TABLE II.

Titration in Mice of the Virus Content of Various Embryonic Organs of the Chick Cultured at 37°C.

Age of culture in days	3			6			9		
Dilution	10^0	10^{-2}	10^{-4}	10^0	10^{-2}	10^{-4}	10^0	10^{-2}	10^{-4}
Whole embryo	D	D	D	D	+	+	D	D	+
Chorio-allantois	D	D	D	D	+	+	D	D	—
Amnion	D	D	D	D	—	—	D	+	—
Yolk sac	—	—	—	D	+	—	D	D	—
Brain	+	—	—	+	—	—	+	—	—
Skin	D	D	+	D	D	+	D	D	+
Lung	D	D	D	D	D	+	D	D	+
Intestine	D	D	+	D	D	+	D	+	+
Heart	+	—	—	—	—	—	D	+	—
Muscle (leg)	—	—	—	+	—	—	—	—	—

Legend same as Table I, except — = mice survived 10 days but had no lung lesions at autopsy.

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[3] Burnet, F. M., *Brit. J. Exp. Path.*, 1940, **21**, 147.

[4] Nigg, C., Crowley, J. H., and Wilson, D. E., *Science*, 1940, **91**, 603.

[5] Burnet, F. M., *Austral. J. Exp. Biol. and Med. Sc.*, 1940, **18**, 353.

[6] Henle, W., and Chambers, L. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 713.

support growth of the virus was investigated in roller tube cultures prepared with fragments of the organ, using the 7-2-1 mixture as nutrient fluid. The tissues suspended in the fluid (taken as a 10^0 dilution) were titrated in mice at 3-, 6- and 9-day intervals. The results are given in Table II.

The virus grew well in all tissues tested except brain, heart and skeletal muscle. In brain tissue the virus apparently survived but did not increase. In skeletal muscle and heart the virus survived but in repeated trials only irregular and small increases in virus content were found. The virus appeared to increase more slowly in yolk sac tissue than in other tissues.

Summary. During a period of 30 days' continuous cultivation of the Melbourne strain of influenza A virus in roller tubes, the respective titers of virus in the chick embryo tissue and fluid components after initial increases remained almost constant, provided the fluid contained serum and embryonic extract. When Tyrode solution or physiological saline was substituted, the amount of virus declined gradually after one week. The virus grew well in cultures of various organs from 14-day chick embryos except in the case of brain, heart and skeletal muscle.

13250

Resistance of Chicks to Infection with Influenza A Virus.

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St. Louis encephalitis virus may be recovered from chicks allowed to hatch after inoculation of the egg[1, 2] but attempts to infect young chicks have resulted only in survival of virus for a limited period without signs of infection.[2, 3] That the body temperature of chicks may be a factor in such resistance to infection has been suggested.[2] Higher temperatures decreased the intensity of reaction of mice inoculated with St. Louis encephalitis virus and of guinea pigs in-

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[1] Harrison, R. W., and Moore, E., *Am. J. Path.*, 1937, **13**, 361.

[2] Sulkin, S. E., Harford, C. G., and Bronfenbrenner, J., *J. Inf. Dis.*, 1940, **67**, 252.

[3] Pearson, H. E., Proc. Soc. Exp. Biol. and Med., 1940, **44**, 413.

fected with endemic typhus.[4, 5] Incubation of eggs at temperatures below 39.5°C favors the growth of some viruses.[6] Thus in Burnet's hands influenza A virus proved lethal or produced lesions in eggs kept at 36°C, whereas neither embryonic death nor lesions occurred at 39°C. Nelson also reported that chick embryos at 28°C reacted less to variola and vaccinia viruses than at 37°C.[7] Furthermore, the multiplication of St. Louis encephalitis virus[8] and lymphogranuloma inguinale viruses[9] in tissue culture has been found to be greater at room temperature than at 37°C. In addition to alteration in body temperature, humoral factors in resistance associated with age have been noted[10] in connection with the reaction of chickens to vaccinia virus. In this paper experiments are described which were undertaken to elucidate the resistance of the young chick to influenza A virus.

The Melbourne strain of influenza A virus passed only in eggs or chick embryo tissue cultures for more than 300 transfers was used. A dilution of 10^{-4} to 10^{-5} of embryo suspension killed all mice after intranasal (i.n.) inoculation of 0.05 cc. The eggs used were from Leghorns except for an occasional one from the Barred Rock variety.

A number of 15-day embryos were inoculated[6] on the chorio-allantoic membranes each with 0.05 cc of 1:500 virus and incubated at 37°C Three were killed after 3 days and the pooled lungs, spleens and intestines were titrated in mice. Enough virus was present in a dilution of 10^{-3} to kill mice and to produce pulmonary lesions in these animals in a dilution of 10^{-5}. Most of the remaining eggs hatched on the 5th day (12 of 39 died but were found to be without definite gross pathology). Three of the chicks were killed immediately after hatching and their pooled organs were tested in mice as described above. No virus was recovered. Additional groups of 3 or 4 chicks were killed at 7, 9, 15 and 19 days after the original inoculation and the lungs and spleens were tested for virus. None was found. The individuals in the 9-, 15- and 19-day groups were bled before death and the respective pools of sera were tested for the

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[4] Lillie, R. D., Dyer, R. E., Armstrong, C., and Pasternack, J. G., *Pub. Health Rep.*, 1937, **52**, 1805.

[5] Castaneda, M. R., *J. Immunol.*, 1937, **33**, 101.

[6] Burnet, F. M., *Med. Res. Council, Spec. Rep. Series*, London, 1936, No. 220.

[7] Nelson, J. B., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 110.

[8] Molloy, E., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 563.

[9] Sanders, M., *J. Exp. Med.*, 1940, **71**, 113.

[10] Duran-Reynals, F., *Yale J. Biol. and Med.*, 1941, **13**, 693.

presence of neutralizing antibodies which were not demonstrated in any case.

When 18-day embryos similarly inoculated were killed after 3 days and the whole embryo (except head and limbs) was tested for virus, irregular results were obtained. Certain of them contained as much virus as was found in 10-day embryos, whereas in others none was revealed by the mouse test.

One-day-old chicks were inoculated, under ether anaesthetic, in the nares each with 0.1 cc of 10% chick embryo virus. Groups of 3 were killed at intervals and their pooled lungs and spleens were tested separately for virus. After 1 and 19 hours, sufficient virus was present in the lungs to kill all mice inoculated with a 10% suspension of those organs. No virus was detected in the lungs at 48 hours nor in the spleens at any of the 3 intervals of time. Two-day-old chicks were similarly inoculated except that the dose was reduced to 0.05 cc of chick embryo virus and the pooled lungs (of 3 or 4) were tested at intervals of 3, 7 and 14 days. No virus was recovered. The chicks killed at 7 and 14 days were bled prior to death and the respective pools of sera were examined for the presence of neutralizing antibodies with negative results. Moreover, antibodies were not found in pooled sera from normal 2-day-old chicks nor from adult chickens.

The ability of the lung tissue from chicks of various ages to support growth of the virus was tested by setting up roller tube tissue

TABLE I.

Titrations in Mice of Influenza A Virus from Roller Tube Cultures (Pooled Tissue and Fluid) of Chick Lung Maintained for Various Intervals of Time at 37°C.

Age of chick	Age of culture (days)	Dilution of culture 10^0	10^{-2}	10^{-4}
18 day embryo	3	D	D	+
	6	D	D	+
	9	D	D	—
	14	—	—	—
20 ” ”	3	D	D	D
	6	—	—	—
	9	+	+	—
	15	—	—	—
2 ” chick	3	D	D	D
	6	—	—	—
	9	D	—	—

D = 50% or more of mice died within 10 days with typical lung lesions.
+ = All mice survived 10 days but had lung lesions at autopsy.
— = All mice survived 10 days but had no lung lesions at autopsy.

cultures of lung in which Tyrode's solution was employed as the nutrient fluid.[11] The results are given in Table 1.

It is evident that tissue removed from chick embryos just before the time of hatching and of 2-day-old chicks supported the growth of virus *in vitro* at 37°C. This finding suggests that no virus-inhibitory property of the tissue, therefore, developed at the time when a mechanism of resistance to infection had been shown to appear.

The body temperature (cloacal) of young chicks was found to be 40° to 41°C and that of adult chickens 41° to 41.6°C. The effect of such temperatures on the propagation of influenza virus in eggs and tissue cultures was studied. The virus was inoculated on the chorio-allantois of 15-day embryos and the eggs then incubated at 41°C. No virus was demonstrated in such embryos when they were tested after 2- and 4-day intervals. Tissue cultures of 15-day embryonic lung and of 2-day chick lung were then set up in roller tubes in the manner mentioned above except that incubation was carried out at 41°C and the tubes were kept stationary. No virus was found in these cultures after intervals of 2, 4 and 6 days despite the fact that the 15-day embryo lung tissue grew fairly well and the 2-day lung tissue grew better at 41° than at 37°C. These data suggest that the high body temperature which presumably becomes established at or about the time of hatching may be the limiting factor in the resistance of chicks to infection with influenza A virus.

Summary. Virus was not recovered after hatching from chicks infected as embryos with the Melbourne strain of influenza A virus. This virus survived and increased in cultures of 18- or 20-day embryo lung and lung tissue from 2-day-old chicks at 37°C, but not in cultures of embryo or chick lung at 41°C. Virus was not recovered from embryos inoculated by the chorio-allantoic route and kept at 41°C. Virus was demonstrated in the lungs of 1-day-old chicks at 19 but not at 48 hours after inoculation in the nares. No neutralizing antibodies for the virus were detected in sera of chicks following such inoculation nor in the sera of normal chicks or adult chickens.

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[11] Pearson, H. E., and Enders, J. F., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 140.

13251

A Study of the Effect of Gramicidin Administered by the Oral Route.*

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(Introduced by Chester S. Keefer.)

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Gramicidin, an extract isolated by Dubos[1] from a spore-bearing soil bacillus, has been found to be bactericidal against gram-positive organisms not only in the test tube but also *in vivo*. Although studies have now been reported on the effect of this substance on the bacterial flora of wounds,[2] no detailed study has been made to determine whether or not gramicidin is capable of altering the intestinal flora through its action in killing the gram-positive components. The purpose of this work, therefore, was to ascertain whether or not this bactericidal agent possessed the ability of changing the intestinal flora of animals from one in which both gram-positive and negative members were present to one in which only the latter could be detected.

Mice were used as the experimental animals. They were fed a diet consisting of Purina Dog Chow which was found to be capable of producing a bacterial flora in the intestine which was rich in *L. acidophilus.* Since this bacterium is gram-positive it should be susceptible to gramicidin. *L. acidophilus* is easily detectable in plates made of a suitable medium by its characteristic colony morphology. Determinations were made at specific intervals of the percentage of the entire viable fecal flora which was made up of *L. acidophilus* before the beginning of the experiment and during the period when gramicidin was fed by mouth. The medium used was a tomato-yeast peptone agar described previously.[3] One stool from each experimental animal was obtained just at the moment of evacuation and emulsified in 5 cc of saline. One-tenth cc of this emulsion was then plated into the tomato agar. The plates were incubated at 37°C for 48 hours and then examined, the percentage of acidophilus colonies being

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* This work was aided in part by a grant from the Lederle Laboratories, Inc., Pearl River, N.Y.

[1] Dubos, R. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 311.

[2] Herrell, W. E., and Heilman, D., *J. Clin. Invest.*, 1941, **20**, 583.

[3] Weinstein, L., Weiss, J. E., Rettger, L. F., and Levy, M. N., *Arch. Int. Med.*, 1933, **52**, 384.

calculated by determining their number in 100 colonies. Before gramicidin was administered 3 determinations of the acidophilus counts were made at intervals of 3 days to establish the level of the organisms in the feces of each of the experimental animals. Fifty mice were used in the experiment, and they were divided into 5 groups of 10 each. The bactericidal agent, tyrothricin,[†] which contains the 2 substances, gramicidin and tyrocidine, was administered in distilled water by means of a ureteral catheter attached to a syringe, the catheter being passed into the stomach. One group of animals was given 0.1 mg of tyrothricin daily for 21 days; a second group was given 0.5 mg daily for the same period; and a third set of animals was fed 1 mg of the bacterial extract daily for an equal length of time. Two groups of mice were kept as controls, one group of 10 receiving 0.1 cc of a 1:10 dilution of 95% alcohol in water to correspond to the alcohol which was administered to the test mice as the vehicle for the tyrothricin, and the other group receiving nothing in addition to the basic diet. In the latter part of the experiment the tyrothricin was fed in the same amounts but was given twice a day, morning and late afternoon.

Results. Studies of the fecal flora prior to the administration of tyrothricin showed that the basal diet was capable of producing a fecal flora which was rich in *L. acidophilus*, some of the animals having counts of this organism as high as 90% of the total viable intestinal organisms. Feeding of the bactericidal agent daily for a period of 3 weeks produced no marked reduction in the number of the aciduric bacteria. The counts on the stools of the experimental animals showed a variation through a range similar to that exhibited by the control mice. Because of the failure to influence the fecal bacteria by feeding tyrothricin in the doses stated once a day, the substance was fed twice a day in corresponding quantities. This procedure, however, did not alter the results and no reduction of the numbers of *L. acidophilus* was detectable.

Since *L. acidophilus* was not killed when tyrothricin was administered by mouth, studies were made of the action of the bacterial extract on this organism in the test tube. Varying amounts of tyrothricin were added to 5 cc amounts of tomato-yeast extract broth and the tubes inoculated with 1 cc of a 48-hour culture of a strain of *L. acidophilus* isolated from one of the experimental animals. The tyrothricin-containing cultures were then incubated at 37° C for 48 hours and 0.2 cc of each mixture plated on tomato-yeast extract agar and examined for typical colonies after 48 hours' incubation. Two different experiments were carried out; it was found that in one instance 0.005 mg and, in the other case, 0.0125 mg of tyrothricin were the largest amounts which would allow *L. acidophilus* to grow; 0.0125 mg and 0.025 mg were found to kill the aciduric organism. These quantities are 1/40 to 1/80 of the largest amount of tyrothricin fed once a day and 1/80 to 1/160 of the largest amount of the bacterial extract fed twice a day.

[†] The tyrothricin used in this study was obtained from Lederle Laboratories, Inc.

One week after the conclusion of the experiment all the animals were killed by stunning; 5 of each group were autopsied and sections of the liver, kidney, stomach, duodenum, jejunum, and colon studied microscopically. All of the tissues were found to be normal and no difference could be detected between the organs of the control animals and those which had been treated with tyrothricin.

Comment. Although *L. acidophilus* was found susceptible to the action of tyrothricin *in vitro* even when small amounts of the bacterial extract were used, this substance had no effect when used *in vivo*. This was true even after the administration of 160 times the quantity capable of killing the organism in the test tube. The reason for this discrepancy is not apparent at the moment, but two possibilities are available in explanation. One is that since the bacterial extract has been shown by Dubos[4] to be a polypeptide, it may be destroyed by the pancreatic or intestinal juices of the animal. Another possible explanation for the failure of tyrothricin to kill *L. acidophilus* in the living animal may be that of the bactericidal agent is inhibited by fecal material; evidence for this hypothesis has been demonstrated in this laboratory.[5]

The failure to produce lesions in the gastro-intestinal tracts of the treated animals may be due to the fact that the tyrothricin was fed in amounts too small to produce such an effect.

Conclusions. Tyrothricin in small amounts is bactericidal against *L. acidophilus in vitro*. Tyrothricin has no effect on the same organism when it is administered by mouth to animals harboring this bacillus even when 160 times the *in vitro* killing dose is given. In the amounts used in these experiments tyrothricin was not found to produce lesions in the gastro-intestinal tract.

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[4] Hotchkiss, R. D., and Dubos, R. J., *Proc. Am. Soc. Biol. Chem.*, 1941, **35**, 63.

[5] Rammelkamp, C. H., and Weinstein, L., unpublished observations.

13252 P

Studies on Radiosensitivity of Animal Cell *in vitro*. I. Radiosensitivity of Muscular and Ciliary Movement.

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Investigations on the effect of X-rays on cells cultivated *in vitro* have been limited with few exceptions to an analysis of irradiation effects on the outgrowth and mitotic activity of cell colonies.[1] Our knowledge of the radiosensitivity of other aspects of cell activity is slight. We considered it justified, therefore, to undertake comparative studies in cell cultures on the sensitivity to irradiation of a variety of cell functions. The following is an account of experiments on the effect of X-rays on muscular contraction and ciliary movement.

Few attempts have been made to influence the kinetic activity of cells and tissues *in vitro* by means of X-rays. (Prime,[2] Gassul,[3] Haberlandt and Sanders,[4] Heeren,[5] Heine,[6] Scott.[7]) In all these experiments no definite inhibiting effect was obtained owing to the small doses employed.

In our experiments we used an X-ray tube which was operated at a tension of ca 35 KV and a current of 30mA with a copper anticathode. The window was aluminium foil 30μ in thickness. Absorption analysis showed that the rays which penetrated through the window and the 0.03 mm thick mica coverglass of the culture were mainly copper-K-rays. The X-ray intensity was about 95,000 r/min at the distance of the irradiated subject.

The following experiments were carried out:

1. *Effect of X-rays on the contraction of heart muscle.* Fragments of myocardium of 7-day-old embryos in hanging drop cultures were used. Irradiation was carried out 2-4 hours after explantation and its effect was observed about 10 minutes after completion of irradiation. A total of 92 heart muscle cultures were irradiated.

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[1] Spear, F. G., *Brit. J. Radiol.*, 1935, **8**, 68.

[2] Prime, F., *J. Canc. Res.*, 1917, **2**, 107.

[3] Gassul, R., *Klin. Wschr.*, 1926, **5**, 560.

[4] Haberlandt, L., and Sanders, R., *Strahlenther*, 1927, **26**, 607.

[5] Heeren, J., *Strahlenther*, 1936, **55**, 293.

[6] Heine, L. H., *Ann. Otology, Rhinol. and Laryngol.*, 1936, **45**, 60.

[7] Scott, C. M., *Some Quantitative Aspects of the Biological Action of X and Gamma Rays*, London, 1937.

To obtain complete and immediate cessation of heart muscle contraction in culture it is necessary to use doses of 500-700,000 r. In 80% of the cultures this result was obtained with 500,000 r, in 15% with 600,000 r and in 5% with 700,000.

2. *Effect of X-rays on the contraction of smooth-muscle.* Fragments of intestine of 18-day-old chick embryos in hanging drop cultures were used. Irradiation was carried out 2-4 hours after explantation and its effect observed 10 minutes after completion of irradiation. A total of 42 cultures were irradiated.

Contractions of smooth muscle cells ceased with doses of 500-900,000 r. In 80% of the cultures doses of 500-700,000 r were sufficient to obtain this effect.

3. *Effect of X-rays on the ciliary movement.* We used freshly explanted ciliated epithelium from the oral cavity of *Buffo viridis.* A total of 80 irradiation experiments were carried out. The ciliary movement was completely arrested by doses of 1-1,400,000 r. Doses of 1,200,000-1,300,000 r arrested movement of cilia in 80% of the cultures.

13253 P

Disappearance of Radioactive Phosphorus from Heart Blood of *Limulus polyphemus.*

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The disappearance of radioactive phosphorus (P^{32}) from the heart blood of the horseshoe crab *Limulus polyphemus,* has been followed in 10 individuals, weighing from 94 to 245 g.

From 0.1 to 0.2 ml of 0.001 molar Na_2HPO_4 solution was injected into the heart at the thoracic-abdominal junction. The amounts of radioactive phosphorus in the solutions and in the samples of blood withdrawn from the heart at frequent intervals following injection were measured with a Geiger-Müller counter, and were expressed as counts per minute per milliliter of solution or per milligram of dried blood. All counts were corrected for background and for decay. The amounts injected were calculated to give equal doses of radioactive phosphorus per gram of body weight, the average dose bearing 3.5×10^5 counts per minute.

In every one of the 10 experiments, the logarithm of the concentration of radioactive phosphorus left in the blood was inversely proportional to time, the slope of the plot relating the two from zero time to 1 hour varying from –0.91 to –3.06. At the beginning of the experiment the average number of counts per milligram of dried blood was 83, and at the end of 1 hour 97.6% of the radioactive phosphorus had disappeared from the blood. A few experiments with the lobster, *Homarus americanus,* gave essentially the same results except that the rate of disappearance was much slower.

These preliminary tests indicate that radioactive phosphorus will be useful in studying various problems in the physiology and biochemistry of the blood and tissues of *Limulus* and other invertebrates, just as it has been in the vertebrates. Several such problems are now being studied.

13254

Liberation of Heparin by Trypsin.

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As pointed out in a preceding report[1] the intravenous injection of trypsin into various animals produces effects remarkably similar to those of anaphylactic shock in the corresponding animals. It was first emphasized by Biedl and Kraus[2] that defective clotting of the blood is a common and conspicuous feature of anaphylaxis in dogs and it has been shown by numerous workers since that delayed coagulation to complete incoagulability of the blood occurs in other laboratory animals during anaphylaxis, although it may not occur so regularly. We have observed the clotting time of the blood in various animals after the intravenous injection of trypsin. We have observed definitely delayed coagulation to complete incoagulability in 8 of 10 dogs after 1 to 2 mg per kilo (Table I) and in 3 of 4 rabbits after 3 to 7 mg per kilo (Table II). We have also observed markedly delayed coagulation in cats, rats and guinea pigs, but the observations do not permit a definite statement as to the frequency of its occurrence in these animals. The development and duration of the coagulation defect were carefully followed in dogs. After the intravenous injection of 1 to 2 mg per kilo, the clotting defect may appear within 2 minutes, is usually well in evidence by 4 minutes, may continue to increase in degree until 10 to 15 minutes and then usually declines gradually so that normal coagulability may be restored anywhere from 20 to 60 minutes after the injection if the animal survives. That this loss of coagulability is not due to a direct effect of trypsin upon the blood is indicated by the fact that corresponding amounts of trypsin added to blood *in vitro* has no appreciable effect upon its clotting ability.

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[1] Dragstedt, C. A., and Rocha e Silva, M., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 420.

[2] Biedl, A., and Kraus, R., *Wien. Klin. Wchnschr.*, 1909, **22**, 363.

TABLE I.
Effect of Intravenous Injection of Trypsin on Clotting Time of Blood in Dogs.

Exp.	Trypsin mg/kilo	Clotting time (min) prior to injection	Clotting time of blood at intervals after injection of trypsin							Reaction
			2′	4′	6′	10′	15′	20′	30′	
I	2*	3		3		3				Mild
II	3*	3	9					3.5		Severe
III	10*	2.5	5							Moderate
IV	5*	2	4							Mild
VII (1)	1.9	3	5							Severe
(2)	2.0	5				30				Died
IX (1)	0.8	2	2				3.5			Moderate
(2)	1.0	3.5	>12	>60						Severe
X	1.0	3		3		>10		5		Moderate
XI (1)	1.2	2		>25		>45		20	4	Severe
(2)	1.4	4	6			>30		>25		”
(3)	1.2	>25		∞		>45				”
XII (1)	2.6	2	3	>10	>20	4				”
(2)	2.6	4	>10	>25						”
XIV (1)	1.0	3	>12		15				4	Moderate
(2)	1.5	4	∞		∞				12	Severe
XV	2.0	3.5		3.5		3.5	3.0			Moderate

*Commercial trypsin. All others—crystalline trypsin (Plaut Lab. Preparation).
∞ Blood incoagulable for more than 60 minutes.

TABLE II.
Effect of Intravenous Injection of Trypsin on Clotting Time of Blood in Rabbits.

Exp.	Normal clotting time, min	Trypsin* mg/kilo	Interval, min	Clotting time, min	Remarks
1	4	4	5	4	Dead in 5 min.
2	4	6	10	>15	Severe shock
3	4	7	3	>24 hr	Dead in 3 min.
4	5	5	7	>60	” ” 7 ”
5	4	12.5	Intravascular	clotting	” ” 2 ”
6	4	25.0	”	”	” ” 2 ”
7†	4	25.0	10	>90	Moderate shock

*Crystalline trypsin (Plaut).
†10 mg heparin injected prior to trypsin.

The nature of the coagulation defect in anaphylaxis has been studied by numerous workers and a great variety of theories have been postulated as to its mechanism. Beginning with the demonstration by Eagle, Johnston and Ravdin[3] of antithrombic activity in the blood of anaphylactized rabbits and dogs, it now appears to be conclusively proved by the work of Waters, Markowitz and Jaques[4] and Jaques and Waters[5] that abnormal amounts of heparin appear in the blood and are responsible for the changes in clotting properties. Their evidence indicates that the heparin is liberated into the blood from the liver. Jaques and Waters made use of the observations of Chargaff and Olson[6] that protamine combines quantitatively with heparin, and found that the coagulability of the blood of shocked animals could be restored to normal by the addition of suitable amounts of protamine. By titrating various samples of blood with varying quantities of protamine, it was possible to give a quantitative expression to the amount of heparin in the blood. We have found that protamine will restore normal coagulability to blood drawn from either rabbits or dogs after trypsin injections and have made quantitative titrations in some of the dog experiments. The observed coagulability of blood samples drawn at various time intervals after the injection of trypsin was roughly parallel to the protamine equivalents of these samples. In 2 experiments peak values of 0.08 and 0.1 mg of protamine per 0.5 cc of blood, respectively, were obtained. These quantities would indicate that a considerable amount of heparin was present as Jaques, Charles and Best[7] found that 1 mg of protamine neutralizes 0.33 mg of heparin.

If the dose of trypsin is increased significantly beyond that which is necessary to produce a severe to fatal reaction, it may produce an extensive intravascular coagulation. As Eagle and Harris[8] have shown, this is due to a direct effect of trypsin upon the blood and can be demonstrated *in vitro* as well as *in vivo*. Intravenous trypsin, therefore, has a dual effect upon the blood, tending to produce an incoagulability indirectly (by the liberation of heparin) and an increased coagulability directly. The mutual antagonism between these effects can be shown in the following ways: The intravascular coagulation which can be produced in a rabbit by the injection of 12 to 25 mg per kilo of trypsin can be prevented by the prior injection of a large dose of heparin (Table II). Likewise, blood which is incoagulable as the result of an injection of 5 mg per kilo of trypsin can be made to coagulate by the addition of further amounts of trypsin. This latter result demonstrates that the loss of coagulability is not due to a destructive action of trypsin upon any of the blood elements essential to its coagulation and indicates that the intravenous injection of shocking doses of trypsin leads to the liberation of sufficient heparin to more than counterbalance the direct coagulant effect of the injected trypsin.

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[4] Waters, E. T., Markowitz, J., and Jaques, L. B., *Science*, 1938, **87**, 582.

[5] Jaques, L. B., and Waters, E. T., *Am. J. Physiol.*, 1940, **129**, 389.

[6] Chargaff, E., and Olson, K. B., *J. Biol. Chem.*, 1937, **122**, 153.

[7] Jaques, L. B., Charles, A. F., and Best, C. H., *Acta Med. Scand.*, Suppl., 1938, **90**, 190.

[8] Eagle, H., and Harris, T. N., *J. Gen. Physiol.*, 1937, **20**, 543.

Summary. The intravenous injection of trypsin in doses of 1 to 2 mg per kilo in dogs, or 3 to 7 mg per kilo in rabbits produces a varying degree of incoagulability of the blood. As the addition of similar concentrations of trypsin to blood *in vitro* does not produce this incoagulability, the latter appears to be an indirect effect. The addition of suitable amounts of protamine restores the coagulability to normal, indicating that the coagulation defect is due to heparin, presumably liberated *in vivo* from various tissues such as the liver.

13255

Effect of Radio-Phosphorus on Blood of Monkeys.*

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Radioactive phosphorus can be administered to animals and man in the form of sodium phosphate in doses sufficient to produce a decrease in the number of white blood cells.[1, 2] In order to obtain further data on the effect of radiophosphorus on the blood of normal animals with respect to the maximum dosage which can be tolerated,

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* This investigation was supported by a grant from the John and Mary R. Markle Foundation.

† We wish to thank the various members of the staff of the Radiation Laboratory, whose generous coöperation makes radio-phosphorus available for biological and medical investigations.

[1] Scott, K. G., and Cook, S. F., *Proc. Nat. Acad. Sci.*, 1937, **23**, 528.

[2] Lawrence, John H., Scott, K. G., and Tuttle, L. W., *The New International Clinics*, 1939, 35-58.

4 young monkeys *(Macacus rhesus)* were given radiophosphorus as a solution of neutral isotonic sodium phosphate intraperitoneally in varying doses. Prior to the administration of radiophosphorus blood samples were collected so that the total red count, white count, and differential count could be determined. Blood samples were taken thereafter at intervals.

Results. An examination of Table I shows that all blood cells drop after administration of radiophosphorus in the dosages used. With respect to the time of maximum drop after radiophosphorus administration it appears that lymphocytes are affected first, the lowest levels appearing 14-21 days in monkey No. 1, 9-13 days in monkey No. 2, and 7-10 days in monkey No. 4. Since monkey No. 3 died from the radiation the lowest level of lymphocytes was observed from 35 to 43 days, a short time before death. The absolute number of granulocytes (polymorphonuclear leucocytes, eosinophiles, and monocytes) reached the lowest levels at a later date: 21-28 days for monkey No. 1, 23-37 days for monkey No. 2, 35-43 days for monkey No. 3, and 22-26 days for monkey No. 4. Red blood cells also were affected at later dates than lymphocytes and were more comparable to granulocytes in this respect.

In these experiments cells presumably originating from the bone marrow are depressed for a longer period of time than lymphocytes. This can be explained on the basis of the greater retention of P_{32} by bone marrow than by other tissues.[2, 4] The excretion of P_{32} by the monkeys used in this work was not measured. However, it can be assumed that radiophosphorus retention in monkeys is similar to that observed in mice and man.[2, 4, 5] After an original retention of 60 to 70% of the total dose, radiophosphorus is slowly excreted in urine and feces (5 to 7% per day loss due to excretion and decay).

A comparison of the percentage of blood cells remaining in monkey blood after P_{32} administration shows that granulocytes and lymphocytes are similarly affected. During the period of maximum effect the percentage of granulocytes in blood decreased to 3, 3, 5, and 2% of the absolute numbers present prior to P_{32} administration. With respect to lymphocytes the absolute number present dropped to 14,

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[3] Lawrence, John H., *Nuclear Physics and Therapy.* Preliminary Report on a New Method for the Treatment of Leukemia and Polycythemia.

[4] Lawrence, John H., and Scott, K. G., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 694.

[5] Lawrence, John H., Tuttle, L. W., Scott, K. G., and Connor, E. L., *J. Clin. Invest.*, 1940, **19**, 267.

TABLE I.
Effect of Radiophosphorus on the Blood of Monkeys (*Macacus rhesus*), counts per mm^3.

Monkey	Radiophosphorus	Days after radiation	Red blood cells (millions)	White blood cells × 100	Granulocytes (absolute number) × 100	Lymphocytes (absolute number) × 100
No. 1	2 M. C. P_{32}	0	6.8	130	74	56
Male. 7 lbs.	1.3 M. C. P_{32}	7	6.6	60	47	13
Sacrificed 28th day.		14	4.6	12	4	8
Grossly tissues		21	4.7	13	2	11
appeared normal.	Sacrificed	28	3.8	24	3	21
No. 2	3.8 M. C. P_{32}	0	5.6	320	150	170
Male. 5 lbs.		9	6.5	37	19	18
		13	6.0	53	46	7
		23	4.8	15	5	10
		37	3.0	28	12	16
		43	4.0	60	28	32
		58	5.0	100	54	46
		102	4.5	156	32	124
		139	5.0	244	64	180
Alive and well.	Wt 7.5 lbs.	436	5.5	131	43	88
No. 3	1.3 M. C. P_{32}	0	6.2	110	40	70
Male. 7.5 lbs.	7.15 M. C. P_{32}	30	5.1	44	18	26
Died 47 days		35	3.8	16	12	4
after P_{32} administration.		43	4.7	12	2	10
Hemorrhagic areas under skin and in intestinal tract. Bone marrow appeared to be entirely hemorrhagic.*	Died	47				
No. 4	4.97 M. C. P_{32}	0	4.8	200	70	130
Female. 7 lbs		7	5.2	43	37	6
at start, 10 lbs		10	5	26	24	2
at end		22	4.2	8	2	6
		26	3.8	6	1	5
		34	2.8	26	21	5
		44	3.0	46	26	20
		56	4.0	58	13	45
		120	4.2	114	38	76
Alive and well.		318	4.3	134	50	24

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*Postmortem examination was carried out 7 hours after death. Microscopic studies revealed the following: *Liver,* no circulating white blood cells. Much edema. *GI tract,* lymphoid follicles absent, practically no cells in submucosa, some interpapillary edema. *Lymph nodes,* absence of normal architecture, follicles frequently devoid of all cells except the stromal cells. *Kidney,* a rare area of cellular degeneration. *Lung,* no significant changes except for some edema. *Spleen,* marked hypocellularity, but normal architecture. *Marrow,* almost complete aplasia, some adult red cell congestion in areas; in general the most important findings were the complete absence of white blood cells in the tissues and the severe aplasia of the marrow and the hypoplasia of the spleen and lymph nodes.

4, 6, and 2% of those present prior to P_{32} dosage (monkeys 1-4 inclusive). Red cells do not appear to be as radio-sensitive. During the maximum effect the red cell count dropped to 47%. Although eosinophiles and monocytes are included in the total granulocyte count, they were identified separately and are affected in the same manner as the polymorphonuclear leukocytes.

Regarding dosage it was found that 1.04 millicuries of radiophosphorus per pound of body weight was fatal (monkey No. 3). However, dosages of .76 and .71 millicuries of radiophosphorus per pound of body weight were tolerated by monkeys No. 2 and No. 4. These monkeys are still alive and appear to be in good health. This level of radiophosphorus dosage would be roughly equivalent to 114 millicuries in one dose to a 150-pound man, this being 10 or more times the dosage used therapeutically in this laboratory. The lethal dosage in small mammals also appears to be comparable. Mice will tolerate as much as 1.36 millicuries of radiophosphorus per pound (unpublished work).

Conclusions. 1. Radiophosphorus lowers the absolute numbers of red cells, lymphocytes, and granulocytes in monkeys. 2. The degree of effect is about the same in lymphocytes and granulocytes, red cells being less affected. 3. Monkeys tolerated dosages of .76 and .71 millicuries of radiophosphorus per pound of body weight, 1.04 millicuries per pound being fatal. 4. The tolerance levels of radiophosphorus are about 10 times that usually used therapeutically.

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Regulation of Skin Temperatures after Lesions of Spinal Nerve Roots.

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The observation has been made that after deafferentation of a hindlimb, the skin temperatures recorded from the footpads of that limb are lower than those in the opposite normally innervated extremity.[1, 2, 3] However, it was found that when such animals were subjected to bilateral abdominal sympathectomy, there was no significant difference in the skin temperatures recorded from the

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[2] Hinsey, J. C., *Am. J. Physiol.*, 1934, **109**, 53.

[3] Zuckerman, S., and Ruch, T. C., *Am. J. Physiol.*, 1934, **109**, 116.

footpads of the two hindlimbs.[4] The conclusion was made that such experiments did not support a tonic reflex control of the blood vessels of the skin via the dorsal roots, as evidenced by skin temperatures in the cat.

The experiments to be reported here represent an accumulation of observations made on cats prepared in our laboratory for various purposes. Dr. Hardy modified his radiometer[5] to make it available for skin temperature studies on animals by reducing the area of skin required for accurate measurement (±0.1°C) to 0.75 sq cm. The animals were examined after having been in a constant temperature room for at least an hour. They were placed in a hammock in such a manner that the circulation to the extremities was not obstructed. After a time, the animals were trained so that they submitted to temperature recordings in a satisfactory manner. This was facilitated by feeding them about 15 minutes before the readings were to be made Readings were recorded until a plateau was reached. Differences of less than 1°C between the corresponding pads of the two sides have not been considered significant.

Three types of experiments were performed: (1) the forelimbs were deafferented, either by section of appropriate dorsal roots or by removal of the ganglia, (2) the hindlimbs were deafferented by section of appropriate dorsal roots, and (3) a considerable portion of the ventral roots to one hindlimb were sectioned. Bilateral sympathectomies were carried out in some of the animals at intervals following deafferentation.

Four animals in which the left forelimbs were deafferented were studied in detail. With the exception of the early postoperative period, the skin temperatures of the left deafferented forepads were not found to be lower than the opposite normally innervated ones, in fact at times they were higher. These observations are illustrated in Figs. 1 and 2. In Cat No. 13, the left dorsal roots were cut from C_4 through T_3 with a resultant anesthesia of the distal portion of the forelimb and yet the temperatures of the forepads of the two limbs were remarkably close together 3 months later (Fig. 1). Following section of the C_8 through T_2 left dorsal roots in Cat No. 3, sensation persisted over the medial surface of the arm as far as the elbow.[6] One month later, there was no significant difference in the pad temperatures in the 2 forelimbs (Fig. 2). Seven days after

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[6] Hare, K., *Am. J. Physiol.*, 1941, **134**, 251.

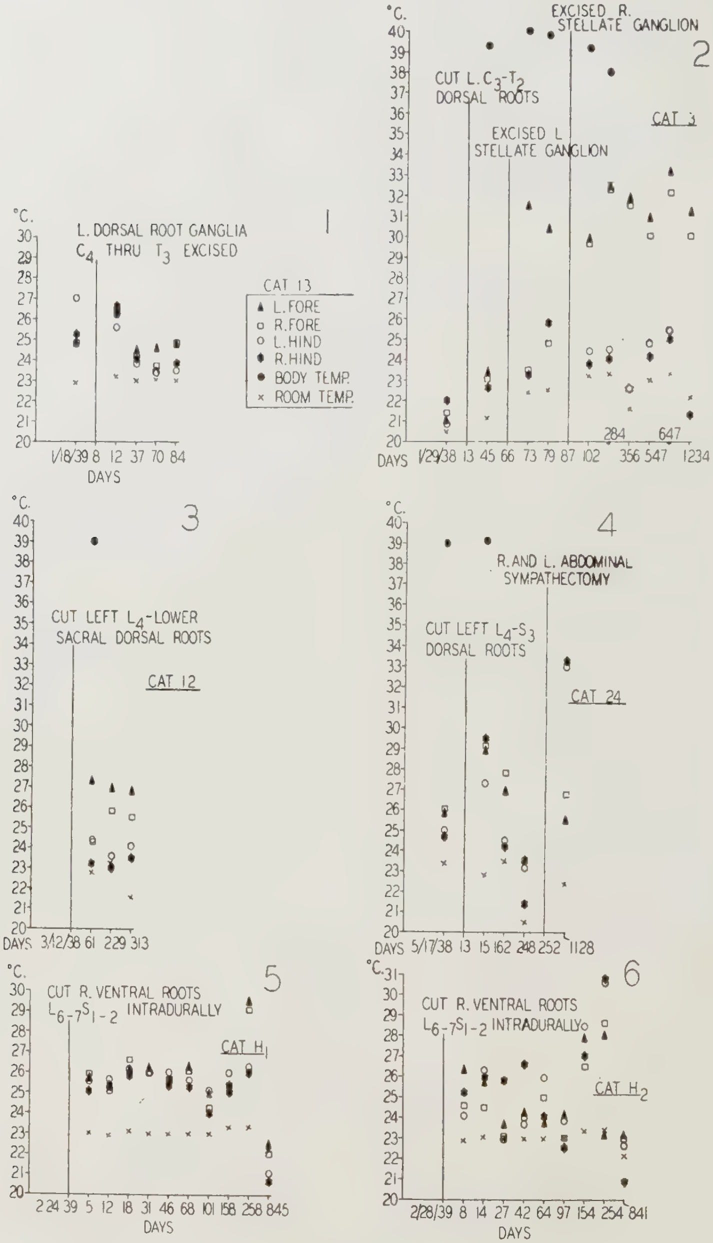

FIG. 1.

removal of the left stellate ganglion, the left forepad was pale and warm (31.5°C) and the right one was pink and cool (23.5°C). Following excision of the right stellate ganglion, the footpads on the 2 forelimbs remained high and close together for a period of 9 months. Later, the deafferented pad was 1°C warmer, possibly due to an abnormal posture of the limb or to some other factor not clear at present. However, the temperatures of the pads of both limbs remained high in comparison with those of the hindlimbs for a period of 3 years after removal of the stellate ganglia.

In Cat No. 12 (Fig. 3), the pad temperature in the 2 hindlimbs were close together 10 months after the deafferentation of the left hind extremity. In Cat No. 24 (Fig. 4), the deafferented pad was colder than the normally innervated one 2 days after the operation. Five months later they were close together, but, after 3 more months, the deafferented pad was warmer. The temperatures on the 2 sides were 33 and 33.3°C 2½ years after bilateral abdominal sympathectomy. This animal has not been necropsied as yet but the chains were probably removed from L_1 through L_7. Apparently, regeneration has not occurred here.[7]

In 3 cats, the 6-7L and 1-2S ventral roots were cut on the right side. At the end of a year, there was sufficient regeneration so that the right limbs were used in walking with little abnormality (Cats H1 and H3). In Cat H2, a marked contracture developed so that the limb was held in extension. By section of these roots below L_5, the thoracolumbar preganglionic fibers escaped[8, 9] and most, if not all, of the somatic motor fibers in the sciatic nerve were separated from their cells of origin.[10] In the presence of paralysis in the distal portion of the right hindlimb, the skin temperatures of the pads on the 2 sides showed no significant differences in 2 of the animals (Fig. 5). In Cat H2, the operated limb showed higher temperatures (1.2 to 2.9°C) for about 5 weeks and then after 8 weeks became significantly colder (1.9° ±) than the normally innervated extremity (Fig. 6). The explanation of the early deviations in temperature is difficult to make unless the operative procedure interfered with a part of the pre- or post-ganglionic supply to the limb. The presence of a contracture later may have contributed to the decreased temperature on the operated side. However, the absence of significant differences in 2 of these animals makes doubtful any visceral innervation to the vessels of the skin by way of the ventral roots distributed to the distal portion of the extremity. Paralysis of the skeletal muscles supplied by the sciatic nerve may not produce measurable variations in the footpad temperatures when comparison is made with the opposite normally innervated side. The experiments demonstrate the validity of the interpretation that the hyperthermia caused by section or crushing of the sciatic nerve is due to interruption of postganglionic nerve fibers coursing in the nerve.[11] Evidence[12] has been presented that such a hyperthermia is restricted to the skin.

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In the presence of substantial evidence for dorsal root reflexes,[13, 14] it is disappointing to fail to find some control of the peripheral blood vessels which would depend upon efferent conduction via the dorsal roots. Although earlier papers presented evidence for such control,[15, 16, 17] more recent work has questioned this interpretation.[18] On the basis of carefully controlled plethysmographic studies on cats and dogs, it has been concluded, "that the existing evidence does not favor the concept of reflex activation of the dorsal root dilators."[19]

The vascular bed may function in the absence of sensory impulses to the local region studied in a manner similar to that in an innervated region. This cannot be interpreted to mean that sensory impulses from a particular region may not have local sign as far as vasomotor reflexes are concerned. In man there are observations which indicate that afferent impulses arising in the leg influence the vasomotor responses of the toe vessels.[20] The absence of sensory impulses may conceivably explain the lower temperatures which have been found in deafferented hindlimbs and the higher temperatures recorded for some deafferented forelimbs. The vascular beds in the pads of 2 corresponding limbs are so similarly distributed that when the thoracolumbar sympathetic pathways on both sides are interrupted and the sensory pathways on one side are cut, the temperatures of the two sides are extremely close together. To be sure, humoral mechanisms are present here but they must be acting upon an anatomical substrate that is closely alike on the two sides.

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Summary. 1. Deafferentation of a cat's forelimb does not lower the skin temperature of the footpad which may remain similar to the contralateral normally innervated one or may be warmer. 2. Deafferentation of a hindlimb may not affect the pad temperature, or it may be lowered or raised. 3. Any differences present in the forelimbs or in the hindlimbs disappear after bilateral sympathectomy. 4. Section of the L_6-L_7 and S_1-S_2 ventral roots of a cat may not significantly affect the pad temperatures.

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Separation of Tobacco Necrosis Virus and Tobacco Mosaic Virus.

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Introduction. During a study of the properties of tobacco necrosis virus, occasional batches of Turkish tobacco plants were encountered which developed not only the non-systemic tobacco necrosis on the lower inoculated leaves, but also through contamination the systemic tobacco mosaic disease. The yield of tobacco mosaic virus (TMV) may be between 2-3 mg per cc of juice,[1] or 100 times that of tobacco necrosis virus (TNV), the average yield of which is about 0.02 mg per cc.[2] Therefore, a single plant carrying a mixed infection in a batch of 100 plants diseased with tobacco necrosis will eventually yield a preparation of TNV containing approximately an equal amount of TMV. In order to avoid the complete loss of TNV in such contaminated preparations, it was considered desirable to attempt the removal of TMV.

TNV is completely soluble over the entire pH range in the absence of large amounts of salt. Since TMV is almost completely insoluble in the region of the isoelectric range, preparations containing very large amounts of TMV were first isoelectrically precipitated. Pirie,

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* Fellow in the Medical Sciences of the National Research Council.

[1] Stanley, W. M., *J. Biol. Chem.*, 1937, **121**, 205.

[2] Cohen, S. S., and Stanley, W. M., unpublished data.

et al.,[3] have demonstrated the absence of cross reaction between TNV and rabbit TMV antiserum. TMV antiserum may, therefore, be used in the quantitative removal of amounts of TMV within the limits of the titer and amount of available antiserum. TNV was finally separated from the lighter serum proteins by sedimentation in the ultracentrifuge.

Although isoelectric precipitation of TMV apparently removed considerable amounts of TNV from solution, the separation by means of antiserum appeared to entail no loss of TNV.

Experimental. Leaves of Turkish tobacco plants having large numbers of necrotic lesions characteristic of tobacco necrosis were harvested 5-7 days after inoculation. The leaves were frozen and stored at −14°C till used. The frozen leaves were ground and allowed to thaw. The juice was expressed at 4°C and a purified virus solution was obtained in the usual manner after at least 4 differential centrifugation cycles, involving a hundredfold reduction in volume. A cycle consisted of ultracentrifugation in the quantity ultracentrifuge at 30,000 rpm for 75 minutes, emulsification of the pellet in water or buffer and centrifugation in an angle centrifuge for 30 minutes at 3000 rpm. The supernatant was then ready for another cycle. All operations were carried out at about 4°C.

Preparations contaminated with TMV were found to have a much greater opalescence than those containing TNV alone. Such preparations were examined in an analytical ultracentrifuge[4, 5] equipped with a Svensson-Philpot[6] optical system. In one case the virus solution before and after treatment with antiserum was examined at 0°C in the Tiselius electrophoresis apparatus equipped with the modification of the schlieren optical system described by Longsworth.[7]

A virus solution containing 1.3 mg per cc TNV and approximately the same amount of TMV was subjected to electrophoresis at pH 6.72 in veronal buffer (0.02 N Na veronal and 0.08 N NaCl). The mobility of TNV (in cm.^{2} volt^{-1} sec^{-1}) was -1.85×10^{-5}; that of TMV was -5.50×10^{-5}. The current was reversed and the boundaries returned to their original positions. To 12 cc of the recovered virus mixture, 2 cc of rabbit TMV antiserum was added. After standing in the cold room overnight, the precipitate was centrifuged off and the solution dialyzed 24 hours against veronal buffer. The solution which was at pH 6.76 was again subjected to electrophoresis. The peak comparable to TNV possessed a mobility of -2.25×10^{-5}. After 3 hours no boundaries other than those of TNV and serum proteins could be detected. It is noteworthy that there is practically no loss of TNV as indicated by the size of the peaks. (Fig. 1.)

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[7] Longsworth, L. G., *J. A. C. S.*, 1939, **61**, 529.

In a second experiment, TMV was first isoelectrically precipitated and small residual amounts subsequently removed with TMV antiserum. Fig. 2 represents the sedimentation of the virus boundaries before and after separation. In this case, the sedimentation constant of TMV was 157×10^{-13} cm/sec/unit field, indicating a concentration of approximately 10 mg/cc.[8] To 10 cc of this virus solution, 0.2 N HCl was added till the pH was 3.5. The solution was placed in the icebox for 1 hour. The supernatant liquid after centrifugation at 3000 rpm for 30 minutes was adjusted to pH 7.4

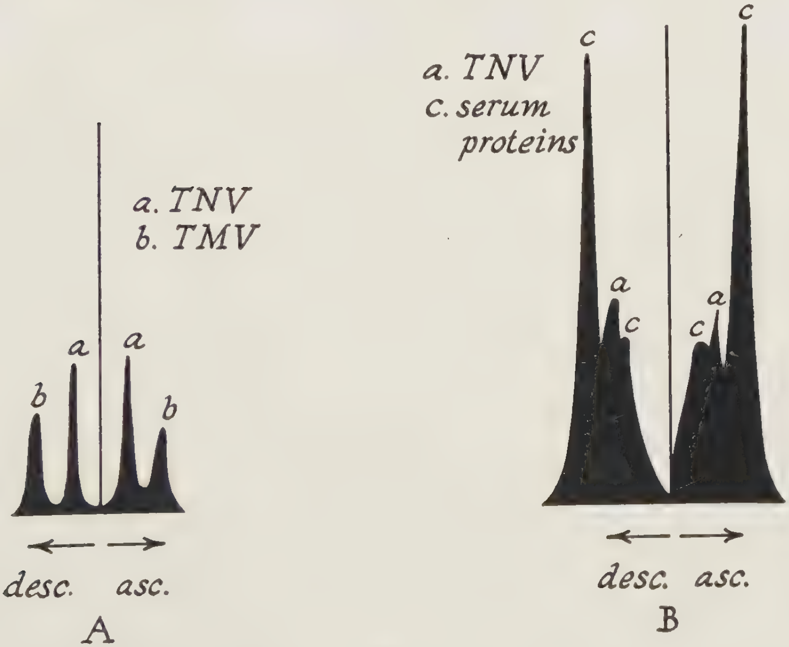

Fig. 1.

Separation of tobacco necrosis virus (TNV) and tobacco mosaic virus (TMV) followed in Tiselius electrophoresis apparatus. A represents boundaries of mixture before separation. B represents boundaries after addition of antiserum to TMV. Boundaries were observed 1 hour after beginning of electrophoresis.

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[8] Lauffer, M. A., *J. Phys. Chem.*, 1940, **44**, 1137.

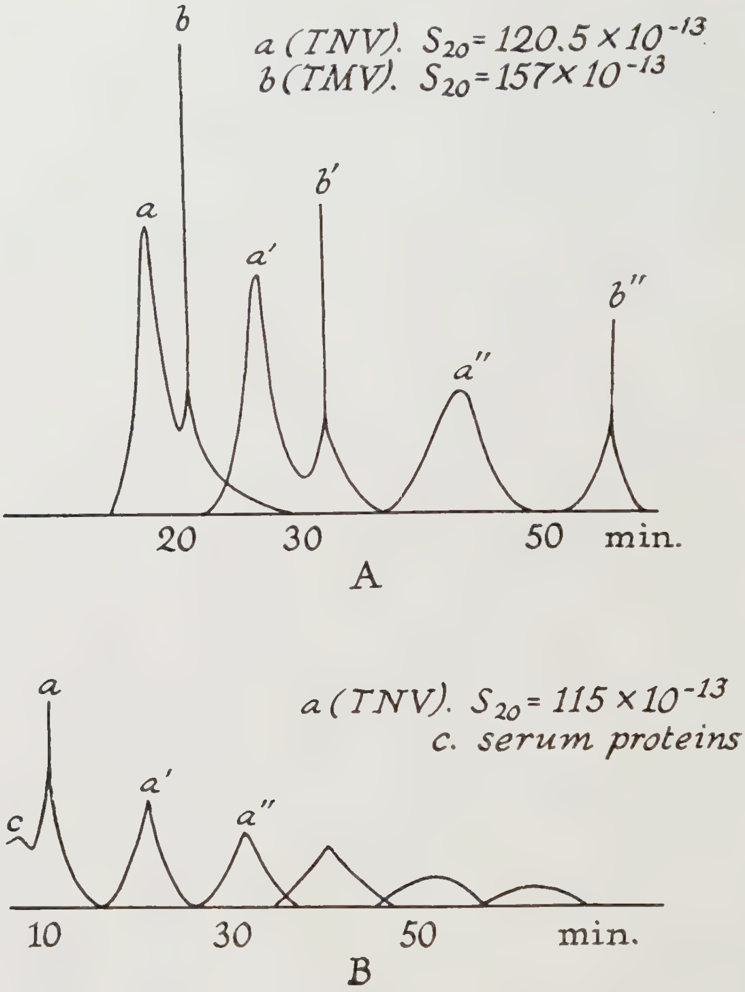

FIG. 2.

Separation of tobacco necrosis virus and tobacco mosaic virus followed in analytical ultracentrifuge. A represents boundaries of mixture before separation. B shows separation of TNV from serum proteins after removal of TMV.

with 0.2 N NaOH. This solution after addition of 0.5 cc TMV antiserum was placed in the icebox overnight. After centrifugation at 3000 rpm for 15 minutes, the supernatant liquid was examined in the analytical ultracentrifuge. The boundary due to TMV had been eliminated.

While the analytical ultracentrifuge and Tiselius apparatus are quite sensitive, it is impossible to detect by means of these instruments concentrations of TMV 100 times greater than is necessary to produce tobacco mosaic in Turkish tobacco. Therefore, the final test of the effectiveness of the separation resided in the determination of the biological activities of the preparations. Before separation, the preparations of mixtures at a concentration of 10^{-5} g/cc protein in 0.1 M phosphate at pH 7.1 caused both tobacco mosaic and tobacco necrosis to develop in Turkish tobacco. After the procedure described above, the same protein concentration produced only tobacco necrosis in Turkish tobacco plants.

Summary. Preparations of mixtures of TMV and TNV were obtained from plants having both diseases. These viruses were then separated by removal of TMV by isoelectric precipitation and by means of absorption with rabbit antiserum to TMV. The separation was followed by means of the analytical ultracentrifuge, the Tiselius electrophoresis apparatus and the biological activities of the preparations.

13258

Assay of the Estrogenic Hormone in Hemophilic and Normal Male Urine.

Carroll L. Birch.* (Introduced by R. W. Keeton.)

From the University of Illinois College of Medicine.

Because hemophilia occurs only in males and is transmitted directly only by females, an assay of the urinary estrogenic hormone was undertaken.

The technic used was identical with that of Dr. Petersen of this institution in order that we might compare the results of our normal control group with his.

White female rats 2 months of age were used. Vaginal smears were made every 12 hours for 3 weeks to ascertain that estrous cycles were normal. Then a laparotomy was performed on each rat under ether anesthesia, the ovaries, broad ligaments and upper part of the cornua of the uterus being removed. The rats were then allowed 10 days to recover from the operation, following which vaginal smears were made at 12-hour intervals for 3 weeks to determine whether the estrous cycles had ceased.

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* These experiments were done under Grant No. 309 from the American Medical Association. The author is very appreciative of this assistance. She is indebted to Miss Helena Petersen (Mrs. Heiple), Mrs. Ruth Christensen, and Miss Alice Bartlett of the Orthopedic Laboratory of the University of Illinois for their untiring technical assistance.

One liter of urine from untreated hemophilic patients was used in each series of experiments. The urine, rendered acid to congo red, was extracted 3 times with benzene in a reflux condenser and evaporated to a syrup. Corn oil was then added and the remainder of the benzene evaporated. This leaves the hormone in the corn oil.

The rats were then divided into groups of 4. Three injections of urine extract of 1/6 cc each were made at 4-hour intervals into each of the 4 rats. Vaginal smears were made at 6-hour intervals for at least 48 hours or until activity ceased. If estrus was induced in 3 out of 4 rats, the test was considered positive.

The number of estrous cycles and the total hours of estrus were determined for the normal and hemophilic groups.

The urine of the first 5 hemophiliacs induced no estrual cycles. These results were recorded in a preliminary report in the *J. Am. Med. Assn.*, Vol. 97, page 244. From this it was suggested that hemophiliacs might be deficient in urinary estrogenic hormone.

A summary of the results of 252 assays on the urine of 52 hemophiliacs and their controls is:

17% of the determinations on hemophilic urines were positive.

28% of the control urines were positive.

The average hours of estrus per positive rat in both groups was 14.

In the control group there was so much variation, not only in different individuals but also in the same individual at different times, that it was decided to assay the urine of 4 individuals for 30 or more consecutive days. This was done to see if there was an orderly sequence in the amount of hormone present. The 4 curves varied widely. Within the time limits of the experiment there was no indication of a regular cycle.

Daily urinary assays were also made for 30 days on 2 hemophiliacs. There was no indication of a regular cycle.

Although the hemophilic group is below that of the normal, the variations in both were so great that the difference is probably not significant.

13259

Effect of Castration and Sex Hormones on Blood of the Rat.

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A sex difference in red cell count has been observed in normal rats,[1] mice,[2] rabbits,[3] cats,[4] dogs,[5] sheep,[6] horses,[7] fowl,[8] and, as is well known, in man.[9] The count, in general, has been found to be higher in the male than in the female, although results contradictory to these have been reported.[10] Castration has been claimed to produce an increase in RBC in female fowl,[11] rabbits,[12] and dogs[13] and a decrease in the male rabbit[14] but such effects have also been denied.[6]

In view of the contradictory nature of the above results and since no detailed experiments have been made on the effects of castration and sex hormones on the blood picture of rodents, it was decided to subject this problem to a more complete investigation in rats.

Materials and Methods. To determine the effects of castration, 10 male and 10 female adult rats (200-255 g) of a highly inbred strain were used. Several preliminary counts were made over a 7-week period preceding the operation. Experimental treatment in the form of daily injections of 10-20 R.U. estradiol† into 6 castrated females and 1-2 mg testosterone† into 6 castrated males was administered for 4 weeks.

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* Accepted in partial fulfillment of the requirements for the degree of Master of Science at New York University.

[1] Adams, E. A., and Shevket, F., *Physiol. Zool.*, 1929, **2**, 181.

[2] Kamenoff, R. J., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 411.

[3] Rosahn, P. D., Pearce, L., and Hu, C. K., *J. Exp. Med.*, 1934, **60**, 687.

[4] Lewis, L. A., *Endoc.*, 1941, **28**, 821.

[5] Otto, J. C., *Arch. f. d. ges. Physiol.*, 1885, **36**, 12.

[6] Storch, A., *Inaugural-Dissertation, Dorpat.*, Karlsruhe, Hacklot, 1901.

[7] Durroux, P. P. D., Thesis No. 140, Bordeaux, 1908.

[8] Riddle, O., and Braucher, P. F., *Am. J. Physiol.*, 1934, **108**, 554.

[9] Ponder, E., *The Mammalian Red Cell and the Properties of Hæmolytic Systems*, Gebruder Borntraeger, Berlin, 1934.

[10] Palmer, C. C., *J. Agric. Research*, 1917-18, **9**, 131.

[11] Blacher, J. J., *Biologia Generalis*, 1926, **2**, 435.

[12] Klieneberger, C., and Carl, W., *Die Blut-Morphologie der Laboratoriums-Tiere*, J. A. Barth, Leipzig, 1912.

[13] Kuhl, P., *Arch. f. d. ges. Physiol.*, 1919, **176**, 263.

[14] Ferrari, R., *Hæmatologica Arch.*, 1930, **11**, 421.

† We are indebted to Dr. Erwin Schwenk, Schering Corporation, for supplies of testosterone propionate and estradiol benzoate.

All determinations were made once or twice weekly from tail blood. Red cell counts, made in duplicate, were required to agree within ±4% and white cell counts to within ±10%. Reticulocytes, stained with brilliant cresyl blue, were counted on wet smears. Differential white cell counts were made on dried smears stained with Wright's. Hemoglobin estimations were performed with a Hellige hemometer. Histological examinations of the bone marrow were made on material obtained from right femurs.

Results. The normal RBC in 50 normal females of our colony

FIGS. 1-4. Graphs showing the mean red cell counts, hemoglobin contents and reticulocyte percentages of 4 groups of rats. Figs. 1 and 2 give the values for 10 animals, Figs. 3 and 4 for 6 animals.

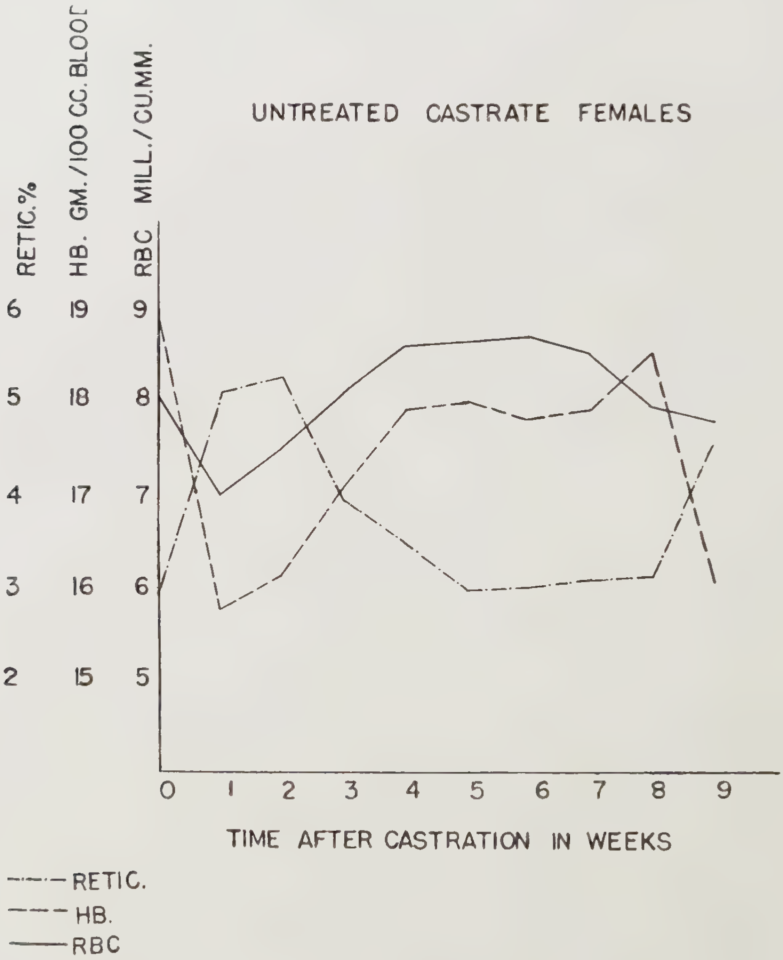

FIG. 1

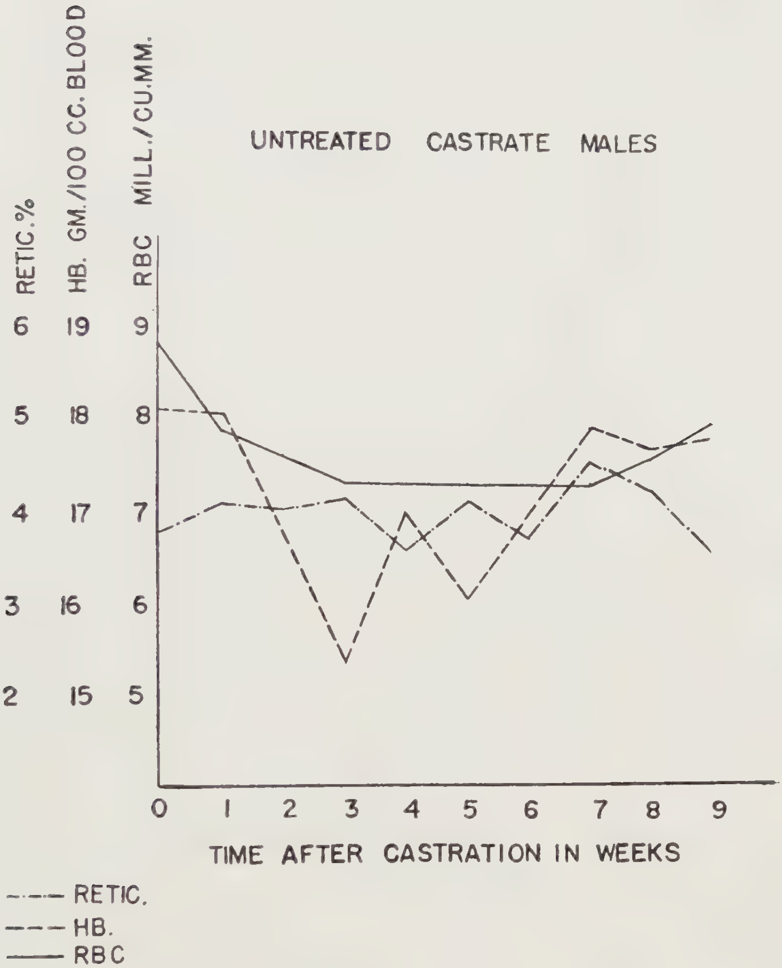

FIG. 2

has been found to be 8.2 ± 0.1 million per mm^3 and that of 25 normal males 8.6 ± 0.1 million per mm^3 As may be seen from Fig. 1, the RBC in the untreated castrated females dropped the first week after the operation and then rose steadily attaining a maximum of 8.8±0.2 million per mm^3 at the end of 6 weeks. Following this, the count tended to drop until the normal count was once again attained at the end of 9 weeks. Reticulocytes rose from 2.9% to a maximum of 5.4% at the end of 2 weeks. There now occurred a steady decrease towards normal values and then a sudden increase to 4.6% at a time corresponding to the fall in RBC.

In the untreated castrated males (Fig. 2), the RBC dropped to 7.4 ± 0.2 million per mm^3 by the end of the 3rd week and remained at this level until the 7th week, after which it tended to rise slightly. Reticulocytes, in males, were unaffected throughout the period of observation.

Injections of estradiol into females, castrated 6 weeks previously, resulted in a drop of RBC from 8.9 ± 0.2 million per mm^3 to 6.7 ± 0.4 million per mm^3 attained at the end of 4 weeks of treatment. Reticulocytes showed a slight increase during this time. In

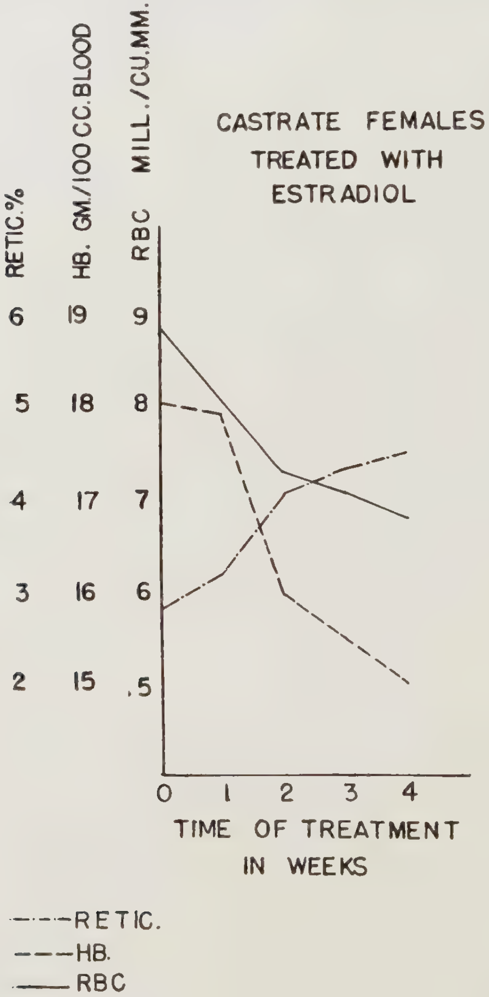

FIG. 3

males, castrated 6 weeks previously, treatment with testosterone caused an increase in RBC from 7.4 ± 0.3 million per mm^3 to an average of 9.1 ± 0.3 million per mm^3 at the end of 4 weeks of injection. Reticulocytes were not affected by this treatment.

A study of the bone marrows indicates a hyperplasia and increased erythrogenic activity in animals injected with testosterone (Fig. 7) or in females not influenced by estrogens—*i. e.,* castrated females (Fig. 6). An erythroid hypoplasia accompanied, in some cases, by

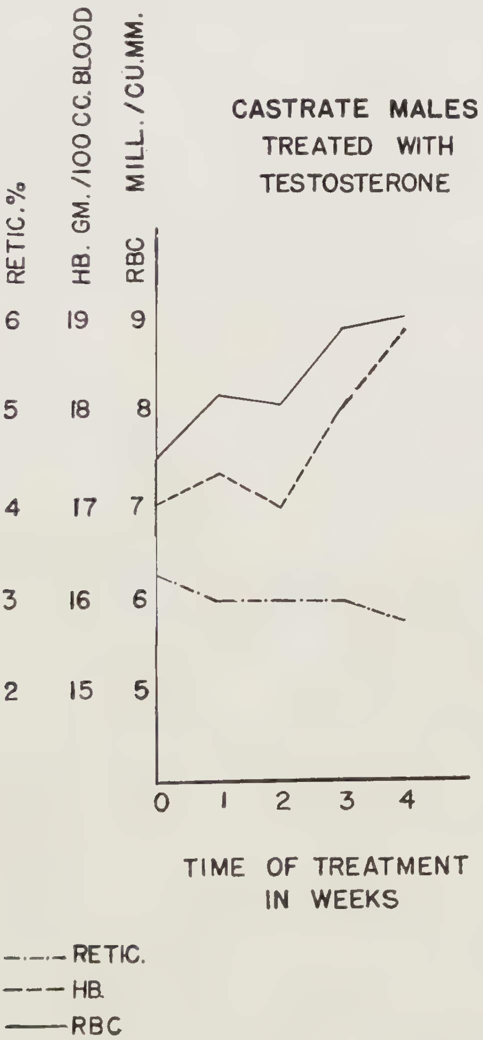

FIG. 4

an increase in the numbers of myeloid areas occurs in the marrows of estrogen-treated animals (Fig. 8) or in those not influenced by androgens—*i. e.*, castrated males (Fig. 5).

Hemoglobin values, in practically every case, seemed to parallel, but were not as marked, as the red cell changes. Variations in white cell counts and differential white cell determinations were too inconstant to warrant any definite conclusions.

Discussion. Normal male rats of our laboratory possess a significantly higher RBC than females. Our data also indicate that the situation is temporarily reversed following castration; the count in the male drops while that in the female rises so that a month after removal of the gonads, the RBC in the male is lower than in the female. It is seen also that the normal sex difference in red cell count may be accentuated with the appropriate sex hormone. Such results may be explained by assuming that androgens stimulate and estrogens depress erythrogenic activity.

FIGS. 5-8. Cross sections of rat femoral bone marrow, fixed in Bouin's and stained with Harris' Hematoxylin-eosin; × 400. FIG. 5—Section from untreated male, 2 months after castration. Note general hypoplasia, diminution in numbers of erythroid elements. FIG. 6—Section from untreated female, 3 months after castration. Some hyperplasia of erythroblastic elements observed. Little vacuolization. FIG. 7—Section from castrated male injected daily for 4 weeks with 1-2 mg testosterone. Intense erythrogenic activity observed. Hypoplasia noted in untreated castrate male (Fig. 5) is repaired. FIG. 8—Section from castrated female injected daily for 4 weeks with 10-20 R.U. estradiol. Vacuolization is marked. Erythrogenic activity is partially inhibited.

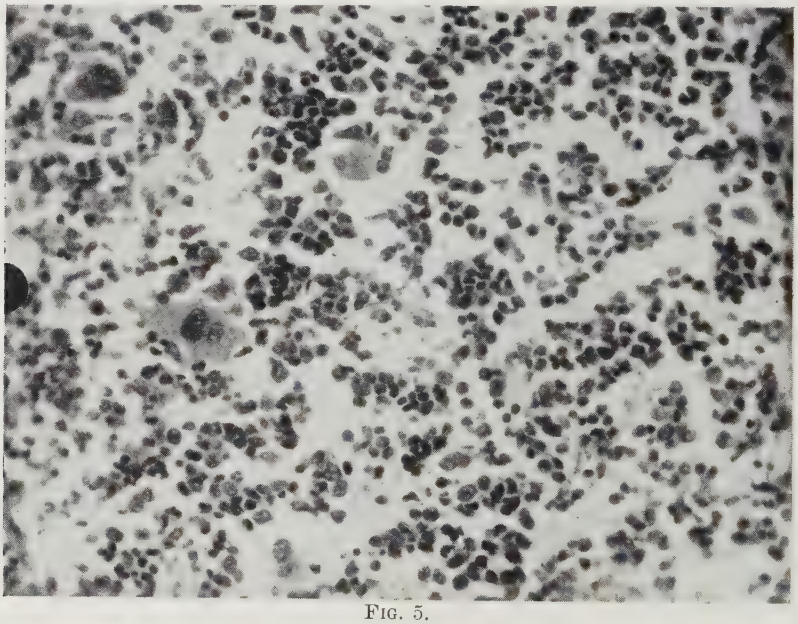

FIG. 5.

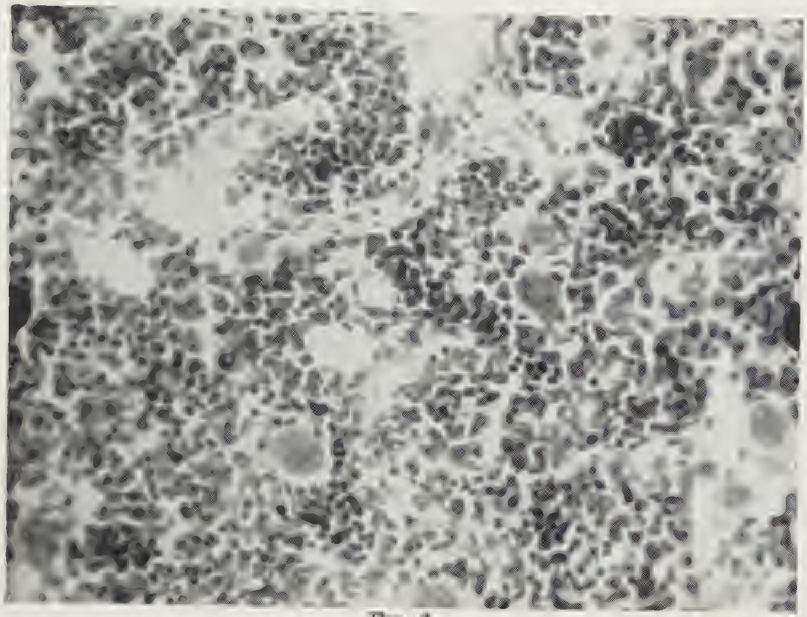

Fig. 6.

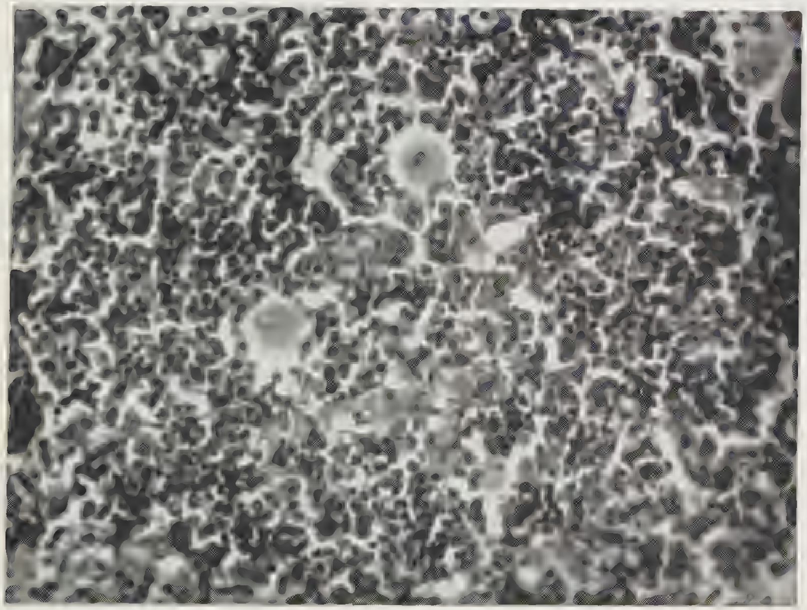

Fig. 7.

That the increase in RBC and hemoglobin in the castrated female rat is due, to some extent, to an increased rate of red cell proliferation may be seen from the behavior of the reticulocytes and the bone

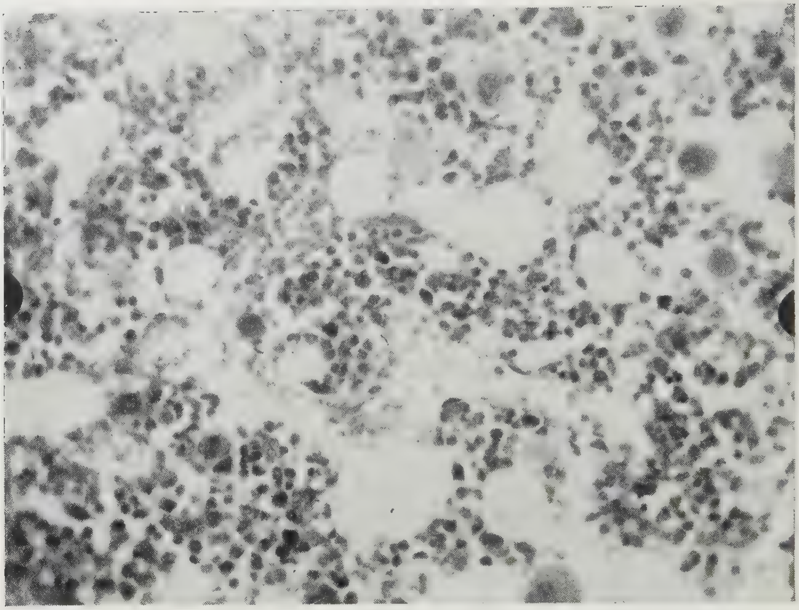

FIG. 8.

marrow appearance. The reticulocytes increase in number soon after the operation, which is to be expected, since, with the removal of the ovaries, estrogenic hormone, which inhibits red cell formation, has been withdrawn. The marrows of untreated castrated females likewise reveal evidence of considerable erythrogenic activity. Moreover, estrogen treatment in castrated females results in marrow hypoplasia, associated with the drop in RBC.

Conversely, castration in the male is attended, for a time, by a diminution in RBC. This is due, most likely, to reduced marrow activity caused, presumably, by withdrawal of testicular hormone. The marrows of untreated castrated male rats show, in fact, a surprising decrease in erythroid areas. The reticulocyte counts remain at relatively low levels in the castrated male which, again, is in accord with the hypothesis set forth above. Although the reticulocyte response to testosterone is not as marked as that observed in the hypophysectomized rat,[15] the influence on the marrow is striking. Myeloid areas become replaced by active red cell progenitors and the general picture is one of erythroid hyperplasia.

The mechanism for the sex hormone influence on marrow activity is not as yet clear. It is unlikely that the sex hormones produce their effects in castrates by operating through the pituitary. One would have to assume, under these circumstances, that estrogens and androgens affect this organ in opposite directions and this is contrary to general evidence. It is possible that the sex hormones influence the blood picture through their well known actions on metabolism and general somatic growth. Estrogens, for example, depress the thyroid which has been shown to be concerned in erythropoiesis.[16] If, however, the thyroid is the only organ concerned, to explain the results, it would have to be assumed that androgens stimulate the production of thyroid secretion, for which there is no direct evidence.

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[15] Vollmer, E. P., Gordon, A. S., Levenstein, I., and Charipper, H. A., *Anat. Rec.*, 1940, **78**, 91.

Summary. 1. The RBC and hemoglobin content of normal rats show higher values than those of normal females. 2. Following castration, the RBC and hemoglobin contents of the males drop, while those in the females rise. 3. Injections of estradiol into castrated females, and testosterone into castrated males produce, respectively, a drop and rise in RBC and hemoglobin. 4. Bone marrow studies indicate that androgens stimulate and estrogens inhibit erythrocytopoiesis. 5. Additional support is thus given to the sex hormone explanation of the normal sex difference in RBC reported for many species of animals.

13260

A New Strain of Virus of Influenza B Isolated During an Epidemic in California.*

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From the Research Laboratory, California State Department of Public Health, Berkeley.

Isolation of strains of influenza virus not antigenically related to the virus of influenza A[1] has been reported by Francis[2] and by Magill.[3] Francis designated his strain influenza B. The virus iso-

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[16] Meyer, O. O., Thewlis, E. W., and Rusch, H. P., *Endoc.*, 1940, **27**, 932.

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation and in coöperation with the California State Department of Public Health.

[1] Horsfall, F. L., Jr., Lennette, E. H., Rickard, E. R., Andrewes, C. H., Smith, W., and Stuart-Harris, C. H., *Lancet*, 1940, **2**, 413.

[2] Francis, T., Jr., *Science*, 1940, **92**, 405.

[3] Magill, T. P., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 162.

lated by Magill was obtained during the same outbreak of influenza in New York, but no data on the antigenic relationship of the 2 viruses have yet been published. The purpose of this paper is to report the isolation of another strain of the virus of influenza B which is antigenically related to, but not identical with, the strain found by Francis.

During January and February, 1940, an outbreak of influenza occurred in California. This epidemic was more or less concurrent with the appearance of influenza in other parts of the United States and the incidence of acute upper respiratory disease of relatively mild character appears to have been quite high. About 8,000 cases were reported from the entire state. Blood specimens were obtained during and after illness from 121 cases of influenza and throat washings were taken on the 2nd to 4th day of illness from 101 of these.

Ferrets were inoculated intranasally with throat washings from 12 cases. One throat washing obtained in the early part of January, 1940, gave an indefinite temperature response in the first ferret and a more marked reaction in the second ferret passage. In 9 subsequent ferret passages a slight rise in temperature was observed in 6 animals on the second or third day after inoculation. Nasal symptoms and loss of appetite occurred in less than half of the ferrets inoculated, but at autopsy the turbinates were usually found to be congested. No definite pulmonary consolidation was seen. At this time four separate attempts to adapt the agent to mice were unsuccessful. Throat washings from many other cases were also inoculated intranasally into mice and onto the chorioallantoic membrane of chick embryos with negative results.

Several months after these observations had been made, Francis announced the isolation of the Lee strain of influenza B. With this strain, which was very kindly sent to us by Dr. T. Francis, Jr., neutralization tests were done on the stored serum specimens from human cases. Fifty out of 76 of the cases of influenza tested showed an increase in antibodies to the Lee strain. In no case was a definite increase in antibodies to influenza A detected. Ferrets inoculated with lung and turbinate material from the 3rd, 6th, 7th, 9th, and 11th ferret passages in the series described in the preceding paragraph were found to have developed antibodies to the Lee strain of influenza B, but had no antibodies to the virus of influenza A (strains PR8, W.S., and 188). It was concluded, therefore, that the ferrets had been infected with a virus antigenically related to the Lee strain. Ferrets inoculated with 11 other throat washings collected during the same epidemic developed no antibodies to influenza A or B.

After the ferret passage virus (referred to as Montgomery strain) had been tentatively identified as influenza B, two subsequent attempts were made to adapt the agent to mice. One of these starting from the 11th ferret passage, was successful and lung lesions appeared in the 11th mouse passage. Some of the mice of the fourth passage although having no lesions were saved for subsequent immunity tests. These were found to be resistant to intranasal inoculation with virus of the 20th mouse passage which killed normal mice in 5 or 6 days with characteristic plum-colored pulmonary consolidation.

In the course of attempts to infect Syrian hamsters *(Cricetus auratus)* and the California kangaroo rats *(Dipodomys deserti deserti* and *Dipodomys merriami simiolus)* with the two strains of influenza B it was noted that these animals developed neutralizing antibodies to rather high titers after intranasal inoculation with mouse passage material.[4] The immune sera from kangaroo rats, hamsters, rabbits, guinea pigs, and ferrets were used in cross-neutralization tests with the Lee and Montgomery strains. In these tests the same suspensions of infected mouse lung kept at constant titer by storage at minus 70°C were used throughout. The titer based on the 50% mortality endpoint of the suspension of the Lee strain was 0.05 cc of a dilution of $10^{-3.05}$ and of the Montgomery strain 0.05 cc of a dilution of $10^{-1.99}$. In the neutralization test a dilution of 10^{-2} or 11 M.L.D. of Lee strain and $10^{-1.30}$or 5 M.L.D. of the Montgomery strain were used with 3 or 4 different dilutions of serum. With the amount of virus used in the tests practically all of the control mice died. In reading the results of the test complete, partial, or no neutralization was recorded by comparing the degree of pulmonary consolidation in the test mice with that in controls according to score method as previously applied.[5]

The results of the cross-neutralization tests are presented in Table I. The titers of sera from animals immune to the Lee strain were approximately 2 to 16 times as high with the homologous strain (Lee) as with the heterologous strain (Montgomery) except with one rabbit serum in which there was no difference. Conversely, the apparent neutralizing capacities of sera from animals immune to the Montgomery strain were 4 to 64 times as high against the

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[4] Eaton, M. D., Martin, W. P., and Talbot, J. C., Proc. Soc. Exp. Biol. and Med., 1941, **48**, 181.

[5] Eaton, M. D., and Pearson, H. E., *J. Exp. Med.*, 1940, **72**, 635.

TABLE I.
Cross-Neutralization with Two Strains of the Virus of Influenza B.

Animal serum No.	Anti-strain	Neutralization of strain									
		Montgomery with serum dilutions					Lee with serum dilutions				
		1:1	1:4	1:16	1:64	1:256	1:1	1:4	1:16	1:64	1:256
Guinea Pig 10-7	Lee	—	P	O	O	O	—	C	P	O	O
Guinea Pig 1-10	Lee	—	O	O	O	O	—	P	O	O	O
Rabbit 3-5	Lee	—	C*	C*	P*	O*	—	C*	C*	C*	O*
Rabbit 3-8	Lee	—	P*	O*	O*	O*	—	P*	O*	O*	O*
Dipodomys 2-9	Lee	—	C	P	P	O	—	C	C	C	P
Dipodomys 6-3	Montgomery	—	C	C	C	—	—	C	P	O	—
Dipodomys 6-7	Montgomery	—	C	C	P	—	—	P	O	O	—
Dipodomys 3-5	Montgomery	C	C	P	O	—	P	P	O	O	—
Hamster 3-54	Montgomery	—	C	C	P	—	—	C	P	P	—
Hamster 3-89	Montgomery	C	P	P	P	—	P	O	O	O	—
Ferret 6-7	Montgomery	—	C	C	C	P	—	C	P	P	O
Ferret 6-8	Montgomery	—	C	C	P	P	—	C	P	P	O
Ferret 2-0	Montgomery	C	C	P	O	—	P	P	O	O	—
Ferret 4-4	Montgomery	C	P	P	P	—	P	O	O	O	—

C Complete neutralization, percentage scores zero (no definite lung lesions).
P Partial neutralization, percentage scores less than half those of controls.
O No definite neutralization, percentage scores more than half those of controls.
— Test not done with serum-dilution indicated.
* Neutralization with 1 M.L.D.

homologous as against the heterologous strain. On the basis of the work of Horsfall and Lennette,[6] these differences in antibody titer could not be accounted for by the small difference in the amounts of the 2 viruses used in the test.

Mice actively immune to the Montgomery strain showed no signs of infection after inoculation with the Lee strain, but were not immune to the virus of influenza A (strain PR8) or to a virus of mouse pneumonia isolated in this laboratory.[7]

Summary. A new strain of the virus of influenza B was isolated from the throat washing of a case of influenza during an epidemic in California in January, 1940. The difficulties of isolating this virus by the usual methods of passage in ferrets and mice are noted. The new strain was not identical in antigenic composition with the Lee strain isolated by Francis, but was closely related to it.

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[6] Horsfall, F. L., Jr., and Lennette, E. H., *J. Exp. Med.*, 1941, **73**, 327.

[7] Pearson, H. E., and Eaton, M. D., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 677.

13261 P

Susceptibility of Kangaroo Rats to Strains of Human Influenza Virus.*

MONROE D. EATON, WALTER P. MARTIN AND JOHN C. TALBOT.
From the Research Laboratory, California State Department of Public Health, Berkeley.

The difficulty of isolating certain strains of influenza virus, especially influenza B, by ferret inoculation has led to a search for more susceptible animals. This paper will report the results of preliminary experiments with 2 species of kangaroo rat *(Dipodomys deserti deserti* and *Dipodomys merriami simiolus)* which were trapped in Southern California and Arizona. Most of the results to be reported were obtained with *Dipodomys merriami simiolus* which appeared to be slightly more susceptible than the other species.

Results with Influenza A Virus. Eight kangaroo rats were inoculated intranasally under ether anesthesia with 10% lung suspension of the 352nd mouse passage of the strain PR8. Two died in 3 and 4 days respectively with massive consolidation of the lungs, 2 survived with consolidation of a considerable part of the lungs, and 4 showed no definite macroscopic lung lesions. Kangaroo rats inoculated with material from the consolidated lungs of the first passage developed only small lung lesions. Virus was demonstrated in the normal appearing lungs of 2 animals by subinoculation of mice and neutralizing antibodies for the strain PR8 were found in the serum of recovered animals. Of 4 kangaroo rats inoculated with the 97th direct ferret passage of strain PR8 none showed definite lung lesions. Two human throat washings demonstrated to contain the virus of influenza A by inoculation of ferrets were instilled into the noses of kangaroo rats and passage of material from the lungs and turbinates was carried out at intervals of 4 to 5 days. Neither lung lesions nor neutralizing antibodies to PR8 were demonstrated in any of the animals. The results indicate that mouse passage strains of influenza A are more virulent for *Dipodomys* than are strains from ferrets or human beings.

Results with Influenza B Virus. The Lee strain which was sent to us by Dr. Thomas Francis, Jr.,[1] (52nd mouse passage) and the

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* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation and in coöperation with the California State Department of Public Health.

[1] Francis, T., Jr., *Science*, 1940, **92**, 405.

TABLE I.
Lung Lesions and Virus in Kangaroo Rats Inoculated Intranasally with Virus of Influenza B.

	Results with Lee strain		Results with Montgomery strain	
Passage No.	lung lesions *Dipodomys*	subinoculations of mice	lung lesions *Dipodomys*	subinoculation of mice
1	±		+,±,±,0	6,7,7,8
2	++,+	+++,++,++,+	±,0,0,0	8,+++,++,++
3	±,0	7,8,8,8	0,0	+,+,+,0
4	+	+++,++,0,0	0,0	8,++,++,+
5	0		0,0	+,+,+,0
6	+,+	3,3,4,4		

Plus signs indicate size of macroscopic lung lesions in each animal; numbers in 3rd and 5th columns day of death of mice with ++++ lung lesions.

Montgomery strain isolated in this laboratory[2] (22nd mouse passage) were inoculated intranasally into a number of kangaroo rats and passages of lung material were done at intervals of 4 to 5 days. The results are summarized in Table I.

The development of lung lesions, particularly with the Montgomery strain, appeared to be irregular, but the presence of the virus in the lungs of infected kangaroo rats can be regularly demonstrated by subinoculation of mice. Neutralizing antibodies to the two strains of influenza B were demonstrated in the sera of kangaroo rats receiving the 1st and 6th *Dipodomys* passages of the Lee strain and the 1st and 4th passages of Montgomery strain.

The ferret passage material of the Montgomery strain which had not been adapted to mice produced no definite lung lesions in kangaroo rats even after repeated passage, but specific neutralizing antibodies were demonstrated in the sera of *Dipodomys* receiving this material. At the time of these studies, no throat washings from human cases of influenza B were available for testing.

Microscopic examination of sections of lungs from *Dipodomys* inoculated intranasally with the viruses of influenza A and B revealed peribronchial infiltration, scant exudate containing red cells and leucocytes in the bronchioles and alveoli, and evidences of desquamation and proliferation of the columnar epithelium in the bronchi. No bacteria, or at most only a few colonies, were grown by culturing the lungs on blood agar.

Although *Dipodomys deserti deserti* and *Dipodomys merriami simiolus* are definitely susceptible to mouse passage strains of the viruses of influenza A and B, further work must be done before definite statements can be made as to the value of these animals for primary isolation of influenza or other respiratory viruses.

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[2] Eaton, M. D., and Beck, M. D., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 177.

13262

Effect of Heat, Cold, Fatigue and Alcohol on Resistance of Mice to Human Influenza Virus.

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In preliminary work it was found that age was an important factor in the resistance of ordinary white mice to the influenza virus. Animals 4 to 6 weeks of age died regularly between 3 to 6 days after 0.05 cc of a 5% influenza filtrate was instilled into their nostrils. Older animals survived the same treatment for longer periods, in some cases as long as 17 days. Studies were outlined in an attempt to learn the effect of heat, cold, fatigue, and alcohol upon the resistance of mice to the virus. However, in early experiments it was found that old animals did not tolerate heat, cold and fatigue; therefore, 4- to 6-weeks-old mice were regularly employed.

The inoculum consisted of 0.05 cc of a 1:100,000 dilution of mouse lung tissue infected with the PR8 strain of influenza virus. It was prepared by grinding a 5% tissue suspension in broth of pH 7.2, centrifugating at 1600 rpm for 10 minutes and then passing the supernatant fluid through a Berkefeld N filter at a negative pressure of 50 cm of mercury. Many experiments were carried out, but only the following will be given since the findings were constant in each instance.

Heat. A group of 9 mice was "hot-room" treated as follows: 6 were inoculated with the virus; the remaining 3 were used as controls. One-half hour after the inoculation, all the animals were placed in the hot room at 37°C and a relative humidity of 100%, for 24 hours. They were transferred to room temperature, 18°C, for 20 hours and then returned to the hot room for 10 hours. This completed the "hot-room" treatment. The animals were sacrificed and autopsied at the end of the fifth day. The data are in Table I.

Cold. A group of 9 mice was "cold treated." Six were given the usual inoculation of virus and the 3 controls were given an equivalent inoculation of nutrient broth. Two hours after the inoculation the mice were thoroughly drenched with cold water and placed in the cold room at 5°C for 5 minutes; they became motionless and cyanosed, and were then placed at room temperature. Two hours later the animals had regained their usual activity. The next day, the process was repeated but the mice were carefully watched and

TABLE I.
Influence of Heat on Resistance of Mice to Influenza Virus.

		Consolidation of lungs*					
			Right				
	Mouse No.	Left	Upper	Middle	Lower	Median	Congestion†
“Hot-Room” Treated + Virus, 1:100,000 Dilution	1	+	0	+	+	+	Medium
	2	+	+	+	+	+	”
	3	+++	0	+	0	0	Extensive
	4	+	0	0	+	+	”
	5	++	+	+	+	+	”
	6	+	+	0	0	+	”
Controls Virus, 1:100,000 Dilution	1	+	0	+	+	0	Slight
	2	0	0	0	0	+++	”
	3	0	+	0	0	++	Medium
Controls “Hot-Room” Treated	1	0	0	0	0	0	Slight
	2	0	0	0	0	0	”
	3	0	0	0	0	0	”

*+ to ++++ = degree of lung consolidation.
†Slight congestion = pinkness of lung gone.
Medium congestion = red lung.
Extensive congestion = “Blood-shot” lung.

kept in the cold room only until they shivered. They were placed at room temperature and observed over a period of 5 days when they were sacrificed and autopsied. The data are given in Table II.

Fatigue. The usual group consisting of 9 mice was fatigued in a slowly revolving treadmill. This instrument was a circular cage 12

TABLE II.
Influence of Cold on Resistance of Mice to Influenza Virus.

		Consolidation of lungs*					
			Right				
	Mouse No.	Left	Upper	Middle	Lower	Median	Congestion†
“Cold-Treated” Mice + Virus 1:100,000 Dilution	1	0	+	++++	0	0	Slight
	2	+	0	0	+	0	Medium
	3	+	0	0	0	0	Extensive
	4	+	+	+	+	++	”
	5	++	+	0	+	++	”
	6	+	+	++	0	+	”
Controls Virus, 1:100,000 Dilution	1	+	0	+	+	0	Slight
	2	0	0	0	0	+++	”
	3	0	+	0	0	++	Medium
Controls “Cold-Treated” Mice	1	0	+	0	0	+	”
	2	0	0	0	+	0	”
	3	0	0	0	+	0	”

*+ to ++++ = degree of lung consolidation.
†Slight congestion = pinkness of lung gone.
Medium congestion = red lung.
Extensive congestion = “Blood-shot” lung.

inches in diameter, made of wire and rotated with an electric motor. During a period of 48 hours before inoculation the mice were exercised a total of 30 hours. The rests were not evenly spaced. However, the mice were thoroughly exhausted at the time of inoculation. Twenty of the 48 hours following the inoculation were spent in the treadmill; rests were evenly spaced. The animals were dispatched and autopsied at the end of 5 days. In one of the duplicate studies certain variations were introduced into the experiment; the mice before inoculation were exercised in the treadmill for 60 hours out of a total of 120. The rest periods again were not evenly spaced. The animals were quite exhausted before inoculation. Of the 48 hours following inoculation, 22 were spent in the treadmill; rests were evenly spaced. The data are given in Table III.

Alcohol. Each of a group of 9 mice was injected intraperitoneally with 0.5 cc of a 10% solution of ethyl alcohol. Five minutes after receiving the alcohol, 6 mice were inoculated as usual with the virus. Three mice remained as controls. As a result of the alcohol treatment the mice were locally irritated and staggered about in their cage for approximately 20 minutes. Twenty-four hours later the treatment with alcohol was again repeated. At the end of 5 days the animals were sacrificed and autopsied. The data are presented in Table IV.

Summary and Comment. On reference to Tables I, II, III, and IV, attention is directed to the amount of virus inoculated: 0.05 cc

TABLE III.
Influence of Fatigue on Resistance of Mice to Influenza Virus.

		Consolidation of lungs*					
			Right				
	Mouse No.	Left	Upper	Middle	Lower	Median	Congestion†
Fatigued Mice + Virus, 1:100,000 Dilution	1	+++	+++	0	+	+	Slight
	2	+++	0	+	+	+	Extensive
	3	+	++	0	+	++	Medium
	4	+	++++	0	+	+	,,
	5	++	+	0	+	++	Extensive
	6	++	0	+	+	0	,,
Controls Virus, 1:100,000 Dilution	1	+	0	+	+	0	Slight
	2	++	0	0	0	+++	,,
	3	0	+	0	0	++	Medium
Controls Fatigued Mice	1	0	0	0	0	0	,,
	2	0	0	0	0	0	,,
	3	0	0	0	0	0	,,

*+ to ++++ = degree of lung consolidation.
†Slight congestion = pinkness of lung gone.
Medium congestion = red lung.
Extensive congestion = "Blood-shot" lung.

TABLE IV.
Influence of Alcohol on Resistance of Mice to Influenza Virus.

		Consolidation of lungs*					
			Right				
	Mouse No.	Left	Upper	Middle	Lower	Median	Congestion†
“Alcohol-Treated” + Virus, 1:100,000 Dilution	1	0	0	0	++	++	Medium
	2	+	0	++	0	++	”
	3	+	+	+	+	+	Slight
	4	++	+	+	0	0	Extensive
	5	+++	+	+	0	+	”
	6	+	++	+	+	+	”
Controls Virus, 1:100,000 Dilution	1	+	0	+	+	0	Slight
	2	0	0	0	0	+++	”
	3	0	+	0	0	++	Medium
Controls “Alcohol-Treated”	1	0	0	0	0	0	Slight
	2	0	0	0	0	0	”
	3	0	0	0	0	0	Medium

*+ to ++++ = degree of lung consolidation.
†Slight congestion = pinkness of lung gone.
Medium congestion = red lung.
Extensive congestion = “Blood-shot” lung.

of 1:100,000 dilution is a sublethal dose. From the results, it is evident that these 4 methods did not materially lower the resistance of the mice to the virus of influenza. Furthermore, fatiguing the group of mice in the one experiment about twice as long as those in the other failed to reveal any difference in the amount of lung consolidation between the two experiments.

This leads to an interesting speculation in the interpretation of the data. Working with experimental pneumococcus pneumonia in rats, Klepser and Nungester[1] were able to demonstrate a distinct influence of alcohol on the incidence of infection. This is in keeping with the oft quoted statement that alcoholism and pneumonia in human beings go hand in hand. The belief on the part of the laity in associating exposure with pneumonia has considerable basis in fact. Yet pneumococcus pneumonia stands out not as an epidemic disease but as an isolated condition. On the other hand, influenza is a distinct epidemic disease. It sweeps through a community, on the basis of the 1918-19 statistics, and infects individuals in its wake regardless of the general physical condition of the individual. Thus, it would appear from observations recorded during epidemics and supported by the data obtained in these experiments that the appearance of influenza in an individual depends on the amount of virus and virulence of the virus rather than the general resistance of the host.

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[1] Klepser, R. G., and Nungester, W. J., *J. Infect. Dis.*, 1939, **65**, 196.

13263

Failure to Produce Neuronal Injury and Necrosis with Poliomyelitis Virus in Rabbits During Insulin Hypoglycemia.*

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Sandler[1] has reported that by depressing the blood sugar of rabbits to 66 mg % or less he was able to produce neuronal injury in the anterior horns with the MV strain of the poliomyelitis virus. The hypoglycemia was thought to decrease cellular oxidation in the tissues of the central nervous system, thus lowering in some unknown manner their susceptibility to the virus. His technic for the demonstration of this phenomenon was as follows: Rabbits were fasted 24 hours, after which they were injected subcutaneously with 0.6 to 0.8 of a unit of protamine insulin per kg of body weight. The hypoglycemia which resulted extended from 3 to 5 hours and sometimes longer. The blood sugar estimations were made by the macromethod of Folin and Wu. During the period of hypoglycemia, the virus was inoculated intracranially, and the fasting was continued. Of the 4 rabbits[2] treated in this manner death occurred in 14 hours, 2 days, 6 days, and 15 days, and histological examination of the spinal cord revealed moderate to severe necrosis of the anterior horn cells.

In the present report the experiments of Sandler were repeated. Normal rabbits were selected, fasted for 24 hours, a blood specimen taken by cardiac puncture, and the animal then injected subcutaneously with 0.6 to 0.8 units of protamine insulin (Lilly) per kg of body weight. After approximately 2 hours, blood was again taken, and the animals were injected intracranially with 1.5 cc of a 10% suspension of monkey cord infected with the MV strain of poliomyelitis virus. If signs of hypoglycemia such as weakness, restlessness or convulsions developed, 10% glucose was injected intravenously. However, several animals died within 10 hours even though sufficient glucose had been administered to raise the level well over 100 mg %. Data on animals lost in this manner are not included in this report.

Seven animals survived for a satisfactory period and had suitable decreases in blood sugar levels. Four of the 7 developed signs of

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* This work was aided by a grant from the Clara Ward Seabury Clinic for the Study of Infantile Paralysis.

[1] Sandler, B. P., *Am. J. Path.*, 1941, **17**, 69.

[2] Sandler, B. P., personal communication.

TABLE I.
Failure to Produce Neuronal Changes in Rabbits by Means of Intracranial Inoculation of MV Virus During Insulin Hypoglycemia.

Rabbit No.	Blood sugar, mg%	Signs of hypoglycemia	Cause of death	Survival period
19*	60	+	Hypoglycemia	18 hr
38†	49	+	”	20 ”
33†	60	+	”	48 ”
42†	61	+	Sacrificed	72 ”
25*	64	0	”	9 days
17*	63	0	”	9 ”
62†	62	0	”	30 ”

*Mixed breed.
†New Zealand white breed, males.

hypoglycemia (Table I). No neurological signs of anterior horn damage appeared, and histological studies of the brain and spinal cord revealed no neuronal injury or other evidence of CNS lesions.[†]

Summary. The intracranial inoculation of rabbits with the MV strain of poliomyelitis virus during insulin hypoglycemia did not produce neurological or pathological signs of anterior horn damage. These findings lend no confirmation to the theory of Sandler that hypoglycemia decreases the cellular oxidation of the central nervous system tissues, thus lowering their susceptibility to neuronal damage by the poliomyelitis virus.

13264

Isolation of Virus from Feces of Mice Receiving Intranasal Inoculations of Epidemic Influenza Virus.

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Epidemic influenza virus has been recovered from the lungs of experimental animals injected intraperitoneally with large amounts of virus.[1] No account of the recovery of the virus from feces has been found in the literature. Accordingly, experiments were outlined to detect the presence of the PR8 strain of influenza virus in the feces of infected animals. At the outset it was essential to develop a method for the treatment of the feces which would destroy the microbic flora without injury to the virus if present. The recent work in which diethyl ether was used under similar conditions with the virus of poliomyelitis suggested investigating this reagent.[2] In a number of experiments of which the following is an example the resistance of the virus to this chemical was clearly demonstrated.

[†] We are indebted to Dr. Konstantin Scharenberg for the histological examination of these tissues.

[1] Rickard, E. R., and Francis, T., Jr., *J. Exp. Med.*, 1938, **67**, 953.

Experiment I. A 5% suspension of infected lung tissue was prepared with infusion broth of pH 7.2. This was centrifugated at 1600 rpm for 10 minutes. The supernatant fluid was passed through sterile Berkefeld N filters at a negative pressure of 50 cm of mercury.

A portion of the filtrate was treated with 10% diethyl ether and kept at 5°C for 90 hours. The ether was then removed by distillation *in vacuo* at 18°C. White mice, 4 to 6 weeks old, which had been sufficiently anesthetized to stop the scratching reflex were inoculated intranasally with 0.05 cc of the ether-treated material.

The animals died in 4 to 6 days after inoculation. At autopsy, their lungs exhibited the same degree of consolidation as the lungs of control animals inoculated with the untreated virus.

Experiment II. On the basis of the forementioned results the experiments were extended in the following manner. Again only typical examples will be given.

A group of 3 mice was inoculated intranasally with 0.05 cc of a 5% virus filtrate. Three days after the inoculation the animals were placed in a clean, sterile cage. Fecal specimens, collected immediately upon defecation, were placed in physiological salt solution and thoroughly emulsified. Enough ether was added to make a 10% mixture which was kept at 5°C for 36 hours. The ether was then removed by distillation *in vacuo* and the residue instilled into the nostrils of 2 mice. Five days later the mice were sacrificed and at autopsy the lungs showed consolidation similar to that caused by influenza virus.

It was essential to establish that the condition in the mice was due to the PR8 strain of virus with which the mice had been infected intranasally. As a consequence, 5 serial passages of the recovered virus were accomplished in mice; the last 2 transfers were by means of 5% filtrates prepared in the usual manner. The animals died within 3 to 6 days after inoculation and at autopsy their lungs were consolidated as in mice dead of influenza. In order to determine the identity of the recovered virus, neutralization tests were employed with 2 samples of PR8 immune ferret sera. These antisera, one specific, the other polyvalent, were secured through the courtesy of Dr. Frank L. Horsfall of the Rockefeller Institute. One cc of each antiserum was diluted 1:5 with physiological salt solution.

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[2] Trask, J. D., Vignec, A. J., and Paul, J. R., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 147.

The following neutralization tests were carried out:

1. *Controls.* (a) Five percent virus filtrate PR8 strain, with specific antiviral serum in a final dilution of 1:10. (b) Five percent virus filtrate, PR8 strain, with polyvalent antiviral serum in a final dilution of 1:10.

2. *Experimental.* (a) Five percent virus filtrate, mouse feces origin, with specific antiviral serum in a final dilution of 1:10. (b) Five percent virus filtrate, mouse feces origin, with polyvalent antiviral serum in a final dilution of 1:10.

The above mixtures were incubated at 37°C for one-half hour; controls to detect any influence of temperature on the 2 virus filtrates were included. Six groups of mice, 3 in each, were inoculated with the 2 filtrates and 0.05 cc of the neutralized mixtures. Aerobic and anaerobic tests for ordinary bacterial contamination were made of the 2 filtrates. No visible growth was evident in any of the filtrates at the end of 4 days.

The groups of mice inoculated with the filtrates died within 3½ to 5 days; in every instance the lungs were completely consolidated. The animals inoculated with the 4 neutralized mixtures were active and healthy 24 hours after inoculation and continued in this state for 6 days, when they were sacrificed and autopsied. No consolidation of the lungs was evident; 2 of the lungs showed slight congestion; 8, medium congestion; and 2 extensive congestion.

Neutralization tests of the influenza virus and the virus filtrates of mice feces origin were not run with normal ferret serum for the reason that it was not available. However, Smith, Andrewes and Laidlaw[3] state in their publications that normal ferret serum does not neutralize influenza virus.

Summary and Comment. 1. The human influenza virus, PR8 strain, is resistant to treatment with 10% ether for at least 90 hours. 2. Influenza virus was present in the feces of mice 3 days after the intranasal inoculation of the virus. This was identified by neutralization tests. 3. In reporting these studies the source of the virus found in the feces has not been considered. There are several possibilities, the most important of which are: (a) The swallowing and survival of part of the material introduced into the nose at the time of the original inoculation. (b) Following multiplication of the virus in the lungs of the animals infected sputa was swallowed. In addition the multiplication of the virus in the digestive tract has been postulated and is being investigated. 4. No attempts have been made to isolate the virus from the feces of human patients with typical epidemic influenza.

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[3] Smith, W., Andrewes, C. H., and Laidlaw, P. P., *Lancet*, 1934, **2**, 66.

13265

Chemical Composition of Fluids from Benign Cysts of the Antrum.

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The maxillary sinus may be the site of several types of benign cysts; this report deals only with the non-secreting type. These cysts form in the connective tissue of the antrum mucosa and are lined by loose connective tissue which contains many capillaries, along with numbers of leucocytes and reticulo-endothelial cells. These cysts vary considerably in size and require a period of weeks or months to develop to the point where they fill the antrum. They contain a clear fluid which coagulates rapidly after removal and has a yellow or amber tinge.

Little is known about the formation of these cysts or why they produce such definite symptoms in a patient. It was thought, therefore, that chemical analyses of the fluid obtained from these cysts might contribute some useful knowledge.

Experimental. The presence of the non-secreting cyst was determined by X-ray films of the sinus, which showed a large, rounded, soft-tissue mass lying in the maxillary sinus. To obtain the fluid from the cyst and to reach the anterior part of the sinus the antrum is punctured with a curved trocar and cannula. Because the cyst may lie anteriorly, the method of puncture must permit exploration of the anterior and inferior region of the sinus as well as posteriorly. The cyst may regress after puncture and lavage. Recurrent or large cysts require surgical removal.

The volume of fluid obtained varied from 2 to 10 cc, depending upon the size of the cyst and the efficiency of the puncture. The specimens were always clear with a yellow tinge and always clotted. The fibrin was removed by twirling on a glass rod and the clear filtrate was used for all analyses. The fluids used for pH and CO_2 determinations were collected under oil.

Cyst fluids were procured from 25 cases. The number of constituents examined depended upon the amount of fluid available. The determinations were rotated to obtain representative data for each constituent. The following determinations were made: pH, total CO_2, water, proteins, non-protein nitrogen, chloride, sodium, potassium, calcium, magnesium, and sugar. The pH was determined colorimetrically.[1] Carbon dioxide was measured with the Van Slyke and Neill manometric gas apparatus.[2] Chlorides were determined by the wet ashing method of Van Slyke[3] with the Wilson and Ball modification,[4] and water by weighing 1 cc aliquots before and after drying to constant weight in platinum crucibles in a 100° oven. Proteins were determined by the micro-Kjeldahl method of Campbell and Hanna[5] and were estimated by multiplying by 6.25, the total nitrogen corrected for non-protein nitrogen.[6] The albumin and globulin were determined by the method of Howe;[7] sodium and potassium were estimated by the methods of Butler and Tuthill[8] and Shohl and Bennett,[9] respectively. Calcium determinations were made by the method of Kramer and Tisdall,[10] as modified by Clark and Collip;[11] magnesium by the method of Denis.[12] For sugar the method of Miller and Van Slyke[13] was applied.

Results. The results may be grouped under 2 heads: (1) Water content and the concentration of glucose and nitrogenous constituents (Table I), and (2) the concentration of electrolytes (Table II).

For comparison with blood serum the analytical data are expressed in units per liter of solute in both media because the average percentage of water in both is approximately the same. From Table I it will be seen that the protein concentration in the cyst fluids was only slightly lower than that found in blood serum. All of the fluids contained fibrinogen. The A/G ratio was also similar to that found in blood serum. The non-protein nitrogen and glucose concentrations were found to be approximately the same as in serum.

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[1] Hastings, A. B., and Sendroy, J., Jr., *J. Biol. Chem.*, 1924, **61**, 695.

[2] Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.

[3] Van Slyke, D. D., *J. Biol. Chem.*, 1923-24, **58**, 523.

[4] Wilson, D. W., and Ball, E. B., *J. Biol. Chem.*, 1928, **79**, 221.

[5] Campbell, W. R., and Hanna, M. I., *J. Biol. Chem.*, 1937, **119**, 1.

[6] Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.*, 1924, **46**, 2066.

[7] Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 93.

[8] Butler, A. M., and Tuthill, E., *J. Biol. Chem.*, 1931, **93**, 171.

[9] Shohl, A. T., and Bennett, H. B., *J. Biol. Chem.*, 1928, **78**, 643.

[10] Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **48**, 223.

[11] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

[12] Denis, W., *J. Biol. Chem.*, 1922, **52**, 411.

[13] Miller, B. F., and Van Slyke, D. D., *J. Biol. Chem.*, 1936, **114**, 583.

The pattern of electrolytes in the fluid (Table II) resembles that of blood serum, the concentration of total base in the fluids never

TABLE I.
Water and Concentration of Glucose and Nitrogen Constituents in Antrum Cyst Fluids.

Patient	H_2O g per l	NPN g per l	Protein g per l	Fibrin g per l	A/G	Glucose mg per l
L.A.	932.0	.32	65.0			
L.B.	933.8	.35	72.5			
G.H.	941.7	.20	60.0	0.81		
T.G.	945.6	.29	57.1	1.78	3.72	
H.B.	946.5	.29	51.0			890
D	955.0					
N.K.	938.8	.22	62.7			
P.D.B.	931.3	.30	67.4		2.05	
R.O.	942.7	.25	63.6		1.87	970
H		.27	54.7	2.18	2.37	950
E.B.						920
J.C.		.26	61.9			970
A.T.						1170
O.L.		.30	49.4		1.95	
S.R.		.30	58.2	0.64	2.48	
Avg	940.8	.28	60.3		2.41	980
	± 6.0	.04	6.4		0.62	60
Serum	937.0	.28	66.6		1.80	920

TABLE II.
Electrolyte Concentration of Antrum Cyst Fluids.

Patient	pH	CO_2 mM per l	Na mM per l	K mM per l	Ca mM per l	Mg mM per l	Cl mM per l
P.D.B.				4.43			101.6
L.A.			149.0	4.55			110.4
L.B.			140.0	3.92	2.25	.90	81.3
G.H.			141.4	5.72	2.45	1.00	109.0
T.G.			138.5	4.50	2.30	.89	103.7
H.B.				3.88			100.2
A.L.	7.64	25.9					
D				4.94			98.6
A.P.			141.8				
N.K.			142.0				103.8
R.O.			139.6	4.19			105.8
J.C.					2.10	.79	92.6
M.N.	7.63	26.55					
E.A.J.	7.67	22.65					
H							98.0
S.R.							98.8
E.B.							102.6
Avg	7.65	25.0	141.8	4.54	2.27	.89	100.5
		±1.8	±3.1	±0.60	±0.12	±.08	±7.2
Serum	7.35	27.5	135.0	4.55	2.70	.81	100.5

being less than that found in blood serum. The sodium content was either equal to that of serum sodium or greater (138.0 mM to 149.0 mM); calcium was generally lower (2.10 mM to 2.45 mM); and potassium and magnesium concentrations corresponded with the normal serum concentrations. The amount of chloride varied from 81.3 mM to 110.0 mM, averaging 100.5 mM. The pH of the three fluids taken under oil was alkaline (7.63-7.67).

Discussion. Photomicrographs show that the collection of fluid in benign cysts of the antrum occurs in the subepithelial connective tissue. There is a distinct similarity between blood serum and cyst fluids from the antrum with respect to the concentrations of certain chemical constituents. The similar concentrations of glucose, non-protein nitrogen, CO_2, calcium, sodium, potassium, and magnesium in the cyst fluid and in normal serum indicate that these diffusible substances pass from the blood to the fluid. The chloride concentrations, which in 7 of the 13 fluids were higher than the average blood serum values, suggest that the cyst fluids are derived essentially from the blood. The protein content, however, is much higher than that of transudates; in fact, it is much like those of true inflammatory exudates. Schade, *et al.*,[14] found total protein values of 3.4 to 5.8 g % in representative inflammatory pleural and peritoneal exudates, which correspond to the magnitude of the total protein (6.0 g %) obtained from the antrum cysts. Since the membranes surrounding the cyst fluids are inflamed and contain many leucocytes and bacteria, it may be assumed that they have become so altered as to permit the free passage of protein with the result that the concentration of this constituent in the effusion is the same as in the blood serum.

Since linear correlations between specific gravity and total solids in serums have been established by Sunderman,[15] and between specific gravity and protein by Moore and Van Slyke,[16] a serum-like protein content in the analyzed antrum fluids should accordingly be accompanied by the high solids and the high specific gravities that were obtained.

As shown above, the pH was on the alkaline side (7.65), and the sugar content was 98 mg %. This corresponds with the pH results of Boots and Cullen[17] who found that non-septic joint fluids were not acid and generally had an alkalinity within the range of normal serum pH; and, also, with the glucose results of Allison and his coworkers[18] who found that the sugar content of non-septic synovial fluids was higher than in infected fluids. Working with sterile synovial fluids with high leucocyte counts Cajori and Pemberton[19] found a high acidity and a low sugar content, but they accounted for this as probably the result of glycolysis. Therefore, the results obtained here indicate that these cyst fluids are sterile and that they do not contain leucocytes in sufficient number to produce glycolysis. Bacteriologically, all of these antrum cyst fluids were sterile by ordinary means of cultivation, which also accounts for the alkaline pH and the normal glucose values.

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[14] Schade, H., Claussen, F., Habler, C., Hoff, F., Mochizucki, N., and Birner, W., *Z. ges. exp. Med.*, 1926, **49**, 334.

[15] Sunderman, F. W., *J. Biol. Chem.*, 1936, **113**, 111.

[16] Moore, N. S., and Van Slyke, D. D., *J. Clin. Invest.*, 1930, **8**, 337.

[17] Boots, R. H., and Cullen, G. E., *J. Exp. Med.*, 1922, **36**, 405.

The above analytical findings, therefore, are analogous to those obtained by previous workers on fluids resulting from transudation from the blood, with the exception of the high protein content. This last semblance to inflammatory exudates may be explained by the increased permeability of the cyst membranes resulting from infection, bacterial products, and proliferative vascular reactions.

Results. Total water, protein, glucose, and electrolyte concentrations were determined on fluids obtained from the non-secreting type of benign cysts of the antrum. The means were as follows: Total water, 940.8, ±0.6 g; protein, 60.3, ±6.4 g; glucose, 980. ±60 mg; pH, 7.65, ±0.02; CO_2, 25.0, ±1.8 mM; chloride, 100.5, ±7.2 mM; sodium, 141.8, ±3.1 mM; potassium, 4.5, ±0.6 mM; calcium, 2.27, ±0.12 mM; and magnesium, 0.89, ±0.08 mM per liter of fluid.

13266

Effect of Digitonin on Solubility of Phospholipids.

H. D. MCEWEN* AND P. L. MACLACHLAN. (Introduced by W. R. Bloor.)

From the Department of Biochemistry and Pharmacology, School of Medicine and Dentistry, University of Rochester, and Department of Biochemistry, School of Medicine, West Virginia University, Morgantown.

In the Bloor-Knudson procedure[1] for the determination of the cholesterol ester content of blood plasma, digitonin in alcoholic solution is added to an alcohol-ether extract of plasma and the mixture evaporated to dryness, thus precipitating the free cholesterol as the digitonide. The cholesterol esters, and presumably the other lipids, are extracted with petroleum ether. However, it was observed by one of us[2] that when phospholipid determinations, by the Bloor oxidimetric procedure,[3] were made on such petroleum ether extracts, no phospholipid was present (Table I). This was interpreted to mean that the phospholipid had been rendered quantitatively insoluble in petroleum ether as a result of the digitonin treatment.

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[18] Allison, N., Fremont-Smith, F., Dailey, M. E., and Kennard, M. H., *J. Bone and Joint Surg.*, 1926, **8**, 748.

[19] Cajori, F. A., and Pemberton, R., *J. Biol. Chem.*, 1928, **76**, 471.

* Present address: School of Medical Sciences, University of South Dakota, Vermillion.

[1] Bloor, W. R., and Knudson, A., *J. Biol. Chem.*, 1916, **27**, 107.

Since blood plasma contains mixed phospholipids it was of interest to determine if lecithin and cephalin could be separately precipitated by digitonin. Experiments to test this were carried out as follows. A sample of mixed phospholipids was prepared from egg yolk from which samples of lecithin and cephalin were obtained by fractionation with absolute alcohol. Aliquots containing known amounts of mixed phospholipid, lecithin and cephalin in petroleum ether solution were evaporated to small volume in 50 cc Erlenmeyer flasks or 15 cc centrifuge tubes and taken up in alcohol. Digitonin in alcoholic solution was then added, carefully mixed, and evaporated to dryness on a steam bath. The residue was extracted with successive portions of petroleum ether and the combined extracts subjected to the Bloor oxidimetric method for phospholipid.

The results (Table I) show that the mixed phospholipid from egg yolk, as well as that in blood plasma, is rendered quantitatively insoluble in petroleum ether by the use of 2 cc of 0.5% digitonin in

TABLE I.
Data on the Precipitation of Phospholipid with Digitonin.

Sample	Amt used mg	Amt recovered %
Using 2 cc of 0.5% digitonin in 50% alcohol		
Mixed phospholipid (blood plasma)	2.08 (4)*	0
” ” (egg yolk)	3.03 (2)	0
Lecithin (egg yolk)	2.94 (2)	0
	2.65 (4)	0
Cephalin (egg yolk)	4.18 (2)	31.2
		28.5
Using 5 cc of 0.2% digitonin in 95% alcohol		
Cephalin (egg yolk)	4.18	1.5
	2.09 (2)	0
” and lecithin (egg yolk)	2.09 1.96	0

*Figures in parentheses = number of samples.

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[2] McEwen, H. D., Thesis, University of Rochester, 1935.

[3] Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

50% alcohol. Lecithin prepared from egg yolk behaves similarly. Although cephalin is completely precipitated when present in mixed phospholipid it is not completely precipitated by this treatment when alone. However, by the use of 5 cc of 0.2% digitonin in 95% alcohol complete precipitation of cephalin was obtained whether alone or when mixed with lecithin.

Summary. It has been shown that mixed phospholipid from blood plasma or egg yolk is rendered quantitatively insoluble in petroleum ether by the use of digitonin. Lecithin and cephalin obtained from egg yolk behave in a similar manner.

13267 P

Effect of Scopolamine on the Fetus.*

Robert Dreisbach and Franklin F. Snyder.

From the Departments of Pharmacology and of Obstetrics and Gynecology, University of Chicago, Chicago, Ill.

The influence of scopolamine on the fetus and on the course of labor is of considerable interest in view of the widespread clinical use of the drug during labor. The clinical observations have been obscured by the simultaneous use of scopolamine with other drugs such as morphine and barbiturates, as well as by numerous complications of labor such as mechanical trauma, anoxemia, and anesthesia. The present experiments represent an attempt to control conditions so that the effect of scopolamine alone may be studied apart from complicating factors.

Cats were selected for study because it was found by trial that cats and dogs react to scopolamine much more readily than rabbits, guinea pigs, and rats.

The effect of scopolamine on the fetus was determined in cats by direct observation of the fetuses following exposure of the uterus by laparotomy carried out beneath the surface of a bath of Ringer's solution at 37°C. Section of the spinal cord in the lumbar region permitted operation without general anesthesia. Several fetuses of a litter were permitted to escape into the bath through incisions of the uterine wall, the umbilical circulation remaining intact. The remainder of the litter was observed within the uterus. The state of activity of the fetuses was carefully noted at frequent intervals during the experiments which lasted from 1½ to 3 hours. Observations were based upon 29 fetuses obtained from 9 litters of which all were at term except one litter at 7 weeks. Dosage of scopolamine was from 10 to 150 mg per kg or a total dose of 30 to 500 mg, which was administered by a single intravenous injection into the maternal animal. Depression of activity was noted in nonpregnant animals after a dosage of scopolamine of 0.25 mg per kg.

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* Aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

The outstanding result of the present observations was the persistence of marked activity of the fetuses despite administration of large doses of scopolamine to the maternal animal. Spontaneous movements and occasional brief intervals of fetal respiratory activity occurred after scopolamine. In several fetuses direct injection of the umbilical vein with 5 to 10 mg of scopolamine resulted in no marked depression of fetal activity. Furthermore the fetuses survived following delivery and were active like controls removed at the beginning of the experiment before injection of scopolamine. An exception occurred in the case of an animal which received 150 mg per kg or a total dose of 500 mg, the fetuses being alive at birth but dying after several hours.

The presence of scopolamine in the fetus following administration to the mother was readily demonstrated by testing samples of fetal urine. Marked mydriasis followed introduction of fetal urine into the conjunctival sac of the cat.

In order to determine the effect of scopolamine on the labor mechanism, the state of the fetuses was observed following spontaneous birth instead of delivery by hysterotomy. In cats at term a daily dose of 1 to 10 mg per kg was injected subcutaneously until parturition occurred. In 4 cats having 15 kittens, all were born alive. In one dog, a primigravida, reared and mated in the laboratory, one mg per kg of scopolamine was injected subcutaneously daily for 5 days preceding parturition. The last injection was made 20 hours before birth. A litter of 11 full-term pups was born within 3 hours. Nine were alive and survived, while the 2 still-born pups were expelled 2 hours after the others.

Conclusion. There was no evidence of fetal injury in cats following administration of large doses of scopolamine to the maternal animal.

13268 P

Effect of Morphine on Labor.*

FRANKLIN F. SNYDER AND KHA TI LIM.

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The occurrence of respiratory depression and failure at birth following the administration of morphine for relief of pain during labor has prompted numerous clinical investigations. Two chief questions arise regarding the mechanism by which administration of morphine to the mother may result in injury of the fetus. There is involved firstly, the effect of morphine upon the fetus directly; and secondly, the effect of morphine upon the labor mechanism. Evaluation of these factors in clinical studies has been difficult because of the complications present at the time of delivery which may result in injury to the child such as anesthesia, anoxemia and mechanical trauma. Accordingly, the present experiments have been undertaken in an attempt to control conditions of observation so that it could be determined whether or not the effect of morphine upon the fetus is of greater consequence than the effect of the narcotic upon the course of labor.

The effect of morphine on the fetus was first studied by direct observation of full-term rabbit fetuses within the unopened uterus according to a method previously described.[1] The change in rate of fetal respiratory movements afforded a sensitive indicator of the depressant effect of the narcotics. Results showed that rhythmical breathing of the fetuses persisted despite administration to the mother of more than 15 times the analgesic dose of morphine.[2]

Additional evidence regarding the state of narcosis of the fetus at various intervals following injection of morphine was obtained in a second series of animals in which the rabbits were not prepared for observation of intrauterine respiration. A dosage of 13 mg per kg of morphine was given by a single intravenous injection in rabbits about the time of the onset of labor, *i. e.*, at 31 or 32 days. This dosage resulted in well marked analgesia of the maternal animal but no loss of consciousness. In 8 litters containing 45 fetuses, all of

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* Aided by a grant from the Committee on Drug Addiction, National Research Council, and the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

[1] Rosenfeld, M., and Snyder, F. F., *Am. J. Obs. and Gyn.*, 1939, **38**, 424.

[2] Snyder, F. F., and Lim, K. T., *J. Pharm. and Exp. Therap.*, 1941, **72**, 39.

the fetuses except one were born alive and survived when delivery was carried out by hysterotomy at intervals varying from 12 minutes to 15 hours after injection. In litters delivered at 12 and 20 minutes after injection, fetal narcosis was marked, while in litters delivered at 12 and 15 hours after injection, the fetuses crawled about actively.

In order to determine the effect of morphine upon the labor mechanism the condition of the fetuses was observed following spontaneous birth. In striking contrast to delivery by hysterotomy, the incidence of stillbirths amounted to 70% when birth occurred spontaneously. In a consecutive series of 92 fetuses obtained from 17 litters, only 28 were born alive while 64 were dead at birth. Prolongation of labor was noted and gave evidence of impairment of the expulsion mechanism. In 13 animals, 13 mg per kg of morphine were given at 31 or 32 days just as in the preceding group delivered by hysterotomy. In the remaining 4 animals a second dose was given after 12 hours.

From the foregoing results it is evident that a reliable method of assay and comparison of drugs used for obstetrical analgesia involves the determination not only of the effect on the fetus but especially of the effect on labor. A sensitive indicator of the effect on the fetus is change in rate of intrauterine respiration. The incidence of stillbirth shows the effect on labor.

When morphine is administered for obstetrical analgesia the occurrence of asphyxia neonatorum has been commonly attributed to the effect upon the fetus of the narcotic. Experiment fails to confirm this. It reveals, on the contrary, that the chief damage of morphine is on the labor mechanism rather than directly on the fetus. In so far as the present experiments reveal the etiology of respiratory failure at birth following administration of morphine during labor, it is evident that injury of the fetus is largely a consequence of injury of the birth mechanism rather than of fetal narcosis.

13269 P

Heart Rate and Respiration Preceding and Following Audiogenic Seizures in the White Rat.

F. Marcuse and A. U. Moore. (Introduced by H. S. Liddell.)

From the Cornell Behavior Farm, Cornell University, Ithaca, N.Y.

In the past 3 years many papers have appeared on the subject of audiogenic seizures. In continuation of previous work[1] it was felt that better insight into the phenomenon would be obtained if a method of recording the physiological factors involved could be devised. This paper describes such a method.

The heart rate was recorded by a Davis electroencephalograph. The 3 electrode leads were soldered to the tops of 3 bicycle valve caps; these caps were held on the animal by suction* applied through tubes leading to each of the caps. The electrodes were applied behind the right and left shoulders and on the midline ventrally. The hair on these areas was trimmed and Cambridge electrode jelly was applied. From these electrodes the heart rate of the animal was recorded (Fig. 1). Respiration (Fig. 1) was recorded by means of a Phipps and Bird accordion type pneumograph, on the tape of the electroencephalograph.

To obtain maximum quiescence during recording, advantage was taken of the rat's fear of height. The animal was placed about 5 feet from the ground with its forefeet and hindfeet on separate perches. These perches were one quarter inch wide and projected 2

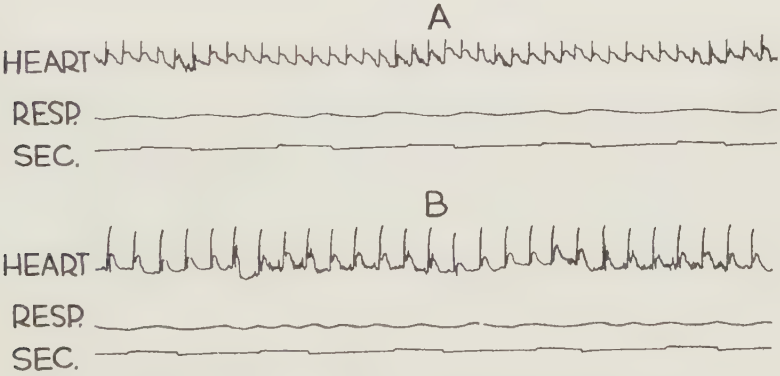

Fig. 1.

A. Heart-rate and respiration preceding an audiogenic seizure.
B. Heart-rate and respiration following an audiogenic seizure.

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[1] Humphrey, G., and Marcuse, F., *Am. J. Psychol.*, 1939, **52**, 616; *J. Comp. Psychol.*, 1941, **32**, 285.

* A small Marathon electric suction tank is run continuously.

inches from the middle of a vertical board. The distance between them could be adjusted to the size of the animal. With this procedure the rat remained almost motionless, and it was possible to adjust the pneumograph around the animal so that it was in contact with the animal only and did not touch any part of the apparatus. The weight of the pneumograph was counterbalanced by a weight on a pulley over the vertical board. This method also facilitated the placing of the ventral electrode.

In Table I are presented the preliminary results concerning heart rate and respiration preceding and following audiogenic seizures in the same animal. The seizure in Rat 2 occurred after 55 seconds of stimulation by a common electric door bell. In Rat 7 the seizure occurred after 25 seconds and was accompanied by ejaculation of semen. Post-seizure records were started approximately 2 minutes after the onset of the passive phase. Data were obtained by sampling the first 5-second interval of every minute. Fig. 1A and 1B are examples of such sampling.

A decrease in heart rate, variability of respiration and an increased range of the indices following audiogenic seizures are indicated.

The short time period required for the onset of a seizure and the ejaculation of semen in Rat 7 suggest, on the basis of previous results, a more susceptible animal. This relationship between the degree of susceptibility to audiogenic seizures and the resultant effects on heart rate and respiration over a prolonged period is at present being investigated.

TABLE I.
Heart Rate and Respiration Before and After Audiogenic Seizures.

Before seizure			After seizure		
Min.	Heart (Rate per min.)	Respiration	Min.	Heart (Rate per min.)	Respiration
			Rat 7.		
4	480	156	1	312	132
3	456	156	2	324	144
2	480	156	3	360	132
1	456	168	4	348	108
Mean	468	159	Mean	336	129
Range	24	12	Range	48	36
			Rat 2.		
4	444	108	1	300	108
3	480	108	2	444	132
2	480	108	3	468	120
1	456	108	4	420	96
Mean	465	108	Mean	408	114
Range	36	0	Range	168	36

13270 P

Phenomenon of Asphyxial Resuscitation. Resuscitation with Inert (Asphyxiating) Gases.

Samuel A. Thompson and George L. Birnbaum. (Introduced by Israel S. Kleiner.)

From the Laboratories of Surgical Research and the Department of Surgery, New York Medical College, Flower and Fifth Avenue Hospitals.

In a study of the various resuscitation procedures in advanced asphyxia, over a hundred experiments were carried out in 43 dogs. During this work, it was observed that in the third phase of asphyxia (cessation of respiration and rapidly falling blood pressure), complete resuscitation of the heart and respiration can be quite regularly produced by rhythmic alternating insufflation and suction with an inert gas (nitrogen, helium), which had just been used to produce the asphyxia. Asphyxial resuscitation was administered at a rate of 15-20 complete strokes per minute, using a positive phase of +14 mm Hg and a negative phase of —9 mm Hg. This resuscitation was continued until the first spontaneous respiration occurred and was then immediately discontinued and the animal allowed to breathe atmospheric air. (Fig. 1.)

The animals were anesthetized by intraperitoneal injection of nembutal (1 cc per 5 pounds). They were then asphyxiated by (1) obstructive (strangulation) asphyxia, or (2) nitrogen, helium, or nitrous oxide inhalation. Either a specially-fitting face mask and pharyngeal airway were used or else a special intratracheal catheter with inflation cuff to prevent leakage.

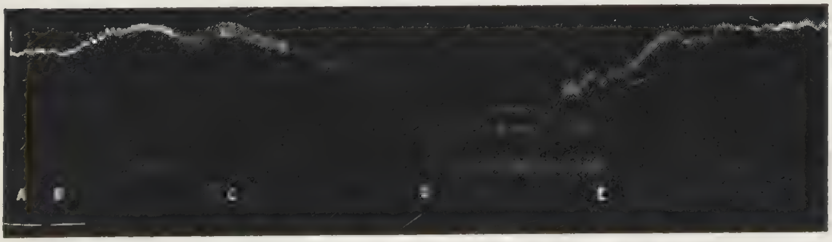

Fig. 1.

Dog 43. *The Phenomenon of Asphyxial Resuscitation.* A, Normal respiration. B, Nitrogen inhalation asphyxia started. C, Respiration has ceased. In the interval C to D, which is 2¾ minutes, the animal's lungs were left connected only to nitrogen gas. D, 2¾ minutes after respiration had ceased, rhythmic inflation and suction of the lungs with nitrogen was started. E, A spontaneous respiration was taken, and the intratracheal tube was opened to the atmospheric air; natural breathing followed.

From above down, blood pressure, respiration, and positive-negative resuscitator.

TABLE I.
Summary of 106 Experiments with Different Resuscitative Procedures.

	Oxygen			Nitrogen		
	Success	Failure	% Success	Success	Failure	% Success
Manual Artificial Respiration	6	5	55	1	6	15
Rhythmic Inflation	7	2	78	2	10	17
” Suction	4	1	80	1	4	20
Resuscitation (Rhythmic Inflation and Suction).	21	1	95	30	5	85

If asphyxia be allowed to reach the third phase (cessation of respiration, rapidly falling blood pressure), the animal ordinarily dies; but if asphyxial resuscitation be applied in this third phase at periods from one-half to 2¾ minutes after cessation of respiration, permanent resuscitation of the heart and respiration can be accomplished quite regularly.

Asphyxial resuscitation, using inert gas, was produced by manual artificial respiration in 15% of the animals, produced by rhythmic positive insufflation in 17%, and produced by rhythmic suction in 20% of the animals. This phenomenon, however, was produced in 85% of the experiments where the combination of rhythmic insufflation and suction was used. (Table I.)

Resuscitation with an inert gas, like resuscitation with oxygen, is more effective the sooner it is applied after cessation of respiration. The phenomenon is abolished or markedly altered by carotid sinus denervation. It is abolished by double vagotomy.

Other physiological studies, including blood gas and alveolar gas analyses, are under way to determine what other factors may be involved in the mechanism of the phenomenon.

13271 P

Continuous and Reproducible Records of the Electrical Activity of the Human Retina.*

LORRIN A. RIGGS. (Introduced by D. B. Lindsley.)

From the Department of Psychology, University of Vermont, Burlington, Vermont.

Two technical problems which have presented considerable difficulty in experimentation on human vision are (1) providing a satisfactory "artificial pupil" through which a beam of light may enter the eye unmodified by the contraction and dilation of the real pupil of the eye, and (2) providing a stable electrical connection with the cornea for the purpose of making continuous records of polarity and action potentials of the retina.

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* Supported by a grant for the necessary apparatus by the National Research Council.

The first of the above problems is met in the present experiments by coating the central portion of a contact lens† with black enamel, leaving an opening of the desired aperture in the exact center of the lens (Fig. 1). This arrangement has the advantage that a diffuse field of light may be used for stimulating the eye. The subject merely sits at a convenient distance from the stimulus field and fixates normally upon a fixation point, without the discomfort of maintaining a rigid posture of the head. Since the artificial pupil moves with the eyeball, small head and eye movements will not interfere with the transmission of the stimulating light to the retina.

The second problem mentioned above, that of maintaining an electrical contact with the cornea, is met by the use of a corneal electrode mounted upon a contact lens as shown in Fig. 1. The electrode consists of a silver disc cemented into a hole in the contact lens. The surface of the disc is flush with the inside surface of the lens. A fine, flexible wire, supported by beeswax, is employed as a lead from the electrode. When the lens is inserted into the eye, the silver makes contact with the physiological saline solution between it and the cornea. A neutral electrode, consisting of a cup filled with

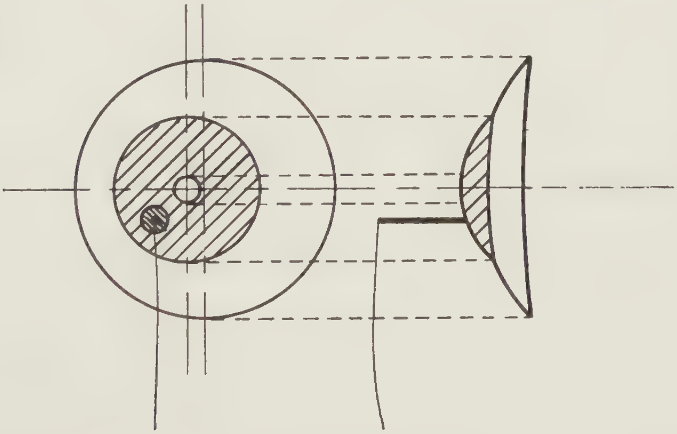

FIG. 1.

Front and side views of a contact lens adapted for use as an artificial pupil and as a mounting for a corneal electrode.

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† The writer is indebted to Dr. R. J. Beitel of the American Optical Company for providing him with a contact lens.

saline, is applied to the skin of the subject over the cheekbone. The stability of these electrodes is such that records may be taken over a period of an hour or more with satisfactory reproducibility.

Fig. 2 presents a sample series of action potential records. These are tracings of photographic records made by a loop oscillograph actuated by amplified potentials from the electrodes described above. Records A to G show the response of the dark-adapted eye to brief (0.15 sec) flashes of "white" light whose relative intensity values are 100, 50, 25, 10, 5, 2.5, and 1, respectively. The short, vertical line below each record signals the moment of stimulation by light. With respect to wave form, the records of this series closely resemble certain of the single records which have been published by investigators who have used a moist thread electrode.[1, 2, 3] Record H is a calibration in the form of an applied potential of 0.25 millivolts lasting for one second.

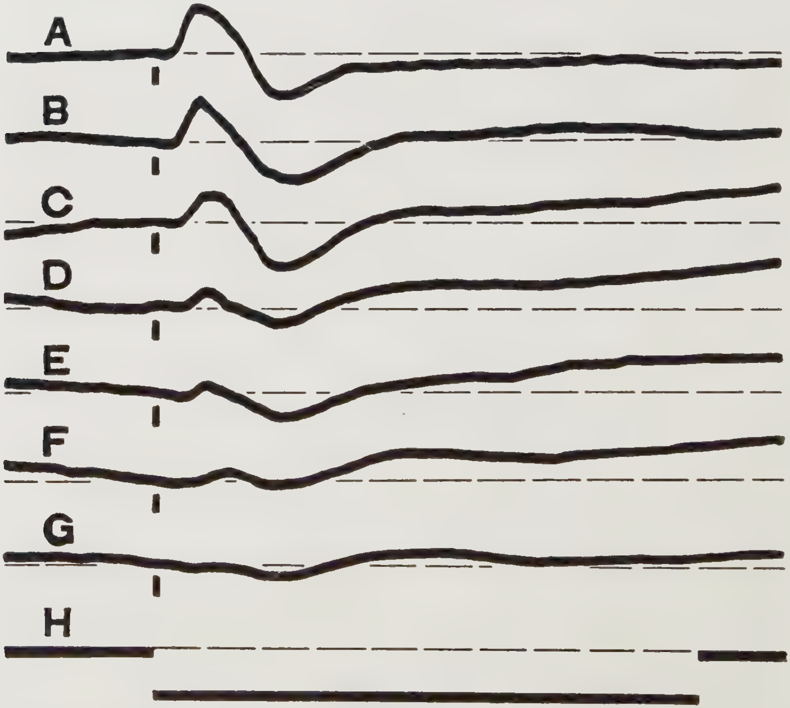

FIG. 2.
A series of electrical responses to brief flashes of light in the dark-adapted eye. (See text for details.)

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[1] Hartline, H. K., *Am. J. Physiol.*, 1925, **73**, 600.

[2] Cooper, S., Creed, R. S., and Granit, R. J., *J. Physiol.*, 1933, **79**, 185.

[3] Gröppel, F., Haass, F., and Kohlrausch, A., *Z. f. Sinnesphysiol.*, 1938, **67**, 207.

By virtue of the present method, which allows for the taking of records over prolonged periods, the writer has recently been able to investigate the form of response obtained during various stages in the process of dark adaptation. Comparative responses of cone and rod receptors have also been studied. It is possible that measurements of the retinal action potential may turn out to be of clinical significance in distinguishing between defects of the retina and those of higher centers.

13272

Cardiac Output in Coronary Occlusion Studied by the Wezler-Boeger Physical Method.*

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The gas analytic methods of determining cardiac output, developed particularly by Grollman, cannot be applied in patients with acute conditions, for example, acute coronary occlusion. We have, therefore, used the physical method of Wezler-Boeger,[1] based upon the equation of the distensibility of the aorta estimated from the pulse wave velocity. The output is calculated from the pulse pressure, pulse wave velocity and aortic diameter. The functional length of the elastic reservoir of the aorta is determined from the natural frequency of the femoral pulse.

$$\text{Stroke Volume (V)} = 2Q\frac{\Delta P \times \frac{a \times T}{4}}{s \times a^2} = \frac{Q}{2} \cdot \frac{\Delta P \times T}{s \times a}\,\text{cc.}$$

Q = Diameter of the aorta, in the outlined cases obtained from Suter's[1] anatomical tables.

ΔP = Pulse pressure in Dyn/cm^2.

T = Natural frequency of the aorta, obtained from femoral pulse tracings.

s = Specific gravity of blood = 1.06.

a = Pulse wave velocity of the aorta, obtained from simultaneous carotid and femoral pulse tracings.

Comparative studies of the Grollman acetylene and the physical methods have been carried out in 70 determinations in 8 normal subjects.[2] Both methods checked within 10%, the absolute values running parallel.

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* Aided by a grant from the American Medical Association.

† Fellow of the Dazian Foundation for Medical Research.

[1] Wezler, K., and Boeger, A., *Erg. d. Physiol.*, 1939, **41**.

[2] To be published.

The cardiac output was determined by the Wezler-Boeger method in 5 patients with acute coronary occlusion during the acute and subacute phases of the attack.

Case One. Hypertension for 7 years. In this case the cardiac output was lowest on the second day of the attack (Fig. 1) at

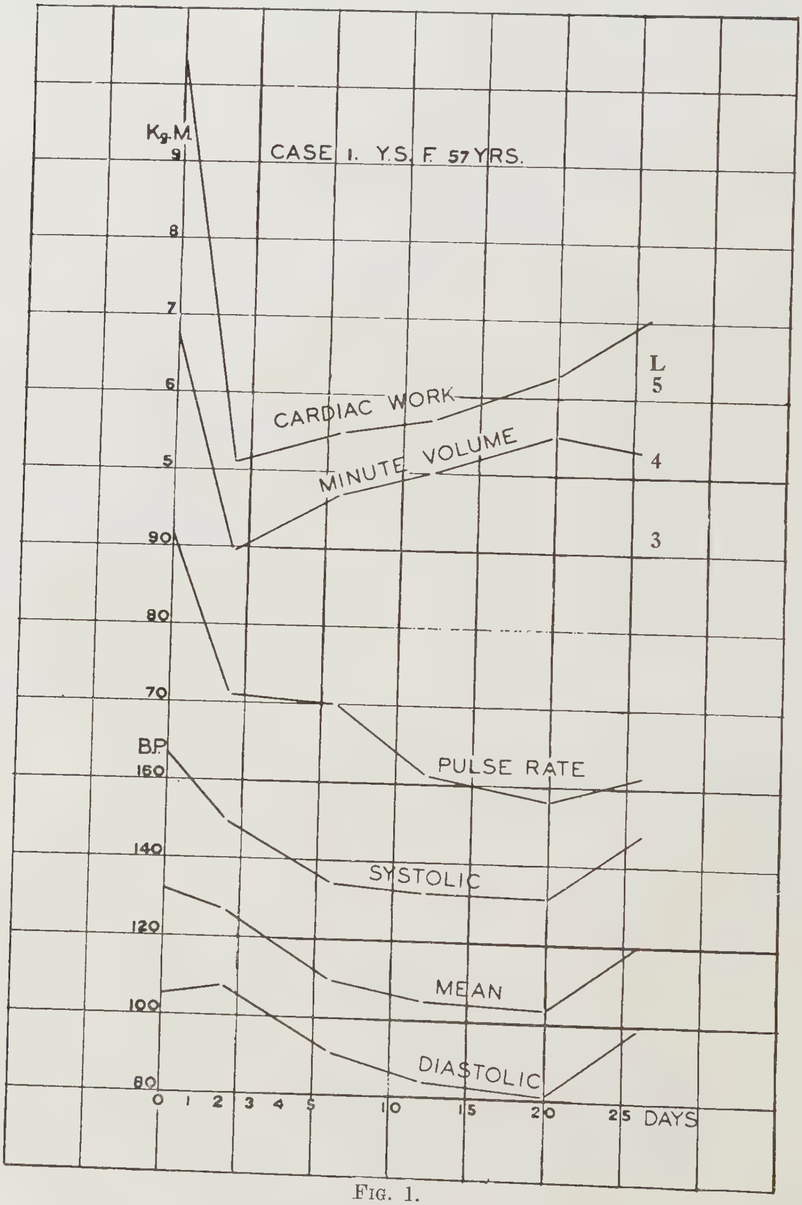

FIG. 1.

which time the systolic, but not the diastolic pressure had fallen, resulting in a diminished pulse pressure. During the following 2 weeks the cardiac output gradually returned to normal. It is im-

TABLE I.

Date	B.P.	P.P*.	Pm	Rate	a in m/sec	S + D	T	V	MVL		Cardiac work KgM/min
Y.S. f.57. Case I.											
2/8/41—6 hr after onset	168/106	62	132	91.6	9.6	.655	.305	63.9	5.9	5.75	10.32
	166/105	61		91.6	9.6	.635	.295	60.9	5.6		
10	151/111	40	127.0	71.7	8.73	.835	.29	42.3	3.0	2.95	5.1
	148/109	39		70.2	8.73	.855	.285	41.4	2.9		
14	136/92	44	109.5	69.4	7.39	.865	.28	54	3.75	3.7	5.5
	132/90	42		70.3	7.39	.85	.285	52.5	3.7		
20	132/85	47	104.6	60.6	7.39	.995	.31	63.9	3.9	4	5.69
	132/83	49		61.2	7.39	.98	.31	66.7	4.1		
3/4	148/99	49	120	61.2	7.62	.98	.335	70.1	4.3	4.3	7.02
B.G. m. 47. Case II.											
4/14 7P.M. 5 hr after onset	200/124	76	156.7	78.5	17.22	.765	.36	46.75	3.7	3.7	7.88
4/15	136/85	51	106.9	69	8.45	.87	.235	41.6	2.9	2.9	4.215
18	132/97	35	111.7	60.3	8.02	.995	.3	38.3	2.3	2.3	3.495
	132/95	37		62.8	7.75	.955	.265	37.1	2.3		
5/5	136/91	45	111.8	66.8	8.16	.9	.33	53.3	3.6	3.55	5.4
	141/94	47		69.8	8.16	.86	.295	49.7	3.47		
21	168/108	60	131	65.3	9.3	.92	.28	52.9	3.5	3.6	6.42
	163/102	61		68.2	9.49	.88	.285	53.55	3.7		
R.W. f. 59. Case III.											
2/8 8 hr	182/121	61	146.7	76.5	11.71	.785	.305	52.4	4	4	7.98
after onset	181/119	63		73.6	11.71	.815	.305	54.2	4		
2/10	148/102	46	123.8	100.15	9.76	.598	.185	26.7	2.7	2.8	4.73
	152/106	46		100.14	10.0	.598	.185	28.2	2.9		
11	124/101	23	110.9	103.3	9.54	.58	.295	17.1	1.8	1.8	2.73
14	116/96	20	107.6	112.1	9.12	.535	.22	15.9	1.8	1.85	2.71
	122/102	20		111.2	8.73	.54	.22	16.6	1.9		
I.M. m. 53. Case IV.											
12/5/40 12 hr	118/88	30	100.5	49.6	7.67	1.21	.53	62.6	3.1	3.15	4.225
after onset	119/86	30		48.3	7.67	1.24	.51	66.1	3.2		
14	128/89	39	105.8	44.8	8.36	1.34	.61	86	3.8	3.8	5.48
	129/91	38	107.3	45.1	8.36	1.33	.62	85.1	3.8		
20	128/88	40	105.2	44.1	8.36	1.36	.625	90.4	4	4	5.17
	130/91	39	107.8	45.4	8.36	1.32	.620	87.5	4		
27	129/87	42	105.1	43.1	8.36	1.38	.61	92.5	4	4	5.83
	128/86	42	104.1	44.75	8.36	1.34	.615	93.3	4.17		
H.W. m. 35. Case V.											
9/17/40 10 hr after onset	135/92	43	110.45	80.5	7.08	.745	.29	43.2	3.5	3.5	5.26
18	112/78	34	92.6	66.7	6.54	.9	.32	40.85	2.75	2.68	3.26
	104/72	32	85.7	68:2	6.54	.88	.315	37.5	2.6		
25	105/70	35	85	64.8	6.54	.925	.315	41.3	2.7	2.7	3.22
	108/72	36	87.9	63.5	6.54	.945	.325	43.2	2.75		
10/24	109/69	40	86.2	54.3	7.08	1.105	.345	47.9	2.6	2.6	2.97
	106/65	41	82.6	52.4	7.08	1.145	.35	49.7	2.6		
	107/66	41	83.6	52.2	6.85	1.15	.32	49.3	2.6		

*P.P.—pulse pressure; Pm—mean pressure; S + D—duration of heartbeat; M.V.L.—minute volume in liters.

portant to note that the cardiac output and blood pressure did not run parallel. Thus the former returned to normal while the blood pressure remained low and did not change later when the blood pressure rose, although the cardiac work was increased.

Case Two. The course in another hypertensive patient was similar to that just described. (Actual number of determinations was 10.)

Case Three. Hypertension for 20 years. The patient sustained a cerebral hemorrhage on the sixth day, after which our observation was discontinued. The patient died of pneumonia on the fifteenth day.

Case Four. Hypertension for 5 years. Coronary occlusion with heart failure. During the stay of 4 weeks in the hospital the cardiac output gradually rose from 3 to 4 liters.

Case Five. Coronary occlusion with complete heart block just before admission. On the fifteenth day the patient developed renal colic with severe shock, which caused a marked drop in cardiac output from 2.7 to 1.7 liters. After several hours the shock disappeared, and the cardiac output returned to the previous level. (Actual number of determinations was 8.)

Summary. In 4 of 5 cases of coronary occlusion the cardiac output fell rapidly, reaching its lowest level between the second and third day. The output began to rise soon after the third day and maintained a relatively constant level after the tenth day. In the 2 hypertensive patients (Cases 1 and 2) the cardiac output rose in spite of the fact that the blood pressure remained low.

Conclusion. The physical method of Wezler and Boeger of determining cardiac output was applied in 5 cases of acute coronary occlusion. The changes in the circulatory dynamics have been analyzed.

13273

Hemolytic Effect of Tyrothricin.*

Charles H. Rammelkamp and Louis Weinstein. (Introduced by Chester S. Keefer.)

From the Evans Memorial, Massachusetts Memorial Hospitals, and the Department of Medicine, Boston University School of Medicine.

A bactericidal agent obtained from a spore-bearing soil bacillus was described by Dubos.[1] The crude extract obtained from the Dubos organism is alcohol-soluble, water-insoluble, and is called *tyrothricin.* From this substance it has been possible to isolate two fractions, *gramicidin* and *tyrocidine,*[2] the former appearing to possess considerably more bactericidal activity both *in vitro* and *in vivo.* Mice which receive intraperitoneal injections of pneumococci or streptococci[1] can be protected completely by small amounts of the crude extract. Tyrothricin, however, will produce definite toxic changes in the animal when injected intravenously[3] into local cavities or intradermally.[4] It has also been observed to cause hemolysis of rabbit's and sheep's erythrocytes *in vitro.*[5]

Heilman and Herrell[6] observed that tyrothricin in amounts as small as 0.001 mg caused hemolysis of rabbit's erythrocytes and that the addition of cholesterol did not neutralize this hemolytic effect. Later, the same authors[7] reported that the hemolytic effect of tyrothricin was due to the presence of *gramicidin.* Dubos and Hotchkiss[5] observed that 0.4 mg of gramicidin did not hemolyze rabbit's erythrocytes suspended in isotonic glucose solution during 24 hours' incubation while tyrocidine, on the other hand, caused some hemolysis in amounts as small as 0.020 mg. In buffer or saline solutions gramicidin was found to produce a slow hemolysis which could be prevented by the addition of small amounts of glucose.

In view of these somewhat conflicting reports it seemed important to study further the hemolytic action of tyrothricin, gramicidin,

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* This work was supported in part by Lederle Laboratories, Inc.

[1] Dubos, R. J., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 311.

[2] Hotchkiss, R. D., and Dubos, R. J., *J. Biol. Chem.*, 1940, **136**, 803.

[3] MacLeod, C. M., Mirick, G. S., and Curnen, E. C., Proc. Soc. Exp. Biol. and Med., 1940, **43**, 460.

[4] Unpublished observations.

[5] Dubos, R. J., and Hotchkiss, R. D., *J. Exp. Med.*, 1941, **73**, 629.

[6] Heilman, D., and Herrell, W. E., Proc. Soc. Exp. Biol. and Med., 1941, **46**, 182.

[7] Herrell, W. E., and Heilman, D., *J. Clin. Invest.*, 1941, **20**, 433.

and tyrocidine, since certain undesirable toxic effects noted during the treatment of human infections may possibly be avoided by using pure gramicidin rather than tyrothricin.

Methods. The tyrothricin,† gramicidin,† and tyrocidine used in these experiments were stored in 95% alcohol in a concentration of 50 mg per cc. Varying amounts of these agents were added to a 5% suspension of rabbit's or human erythrocytes in (a) 0.85% saline, (b) 5 and 10% glucose in distilled water, and (c) 5 and 10% glucose in 0.85% saline, the total volume in each tube being 2 cc. Controls using similar dilutions of 95% alcohol were made in each instance. The tubes were then incubated at 37°C and readings for hemolysis made at 1, 3, 5, and 24 hours.

Results. Table I shows the results obtained in one experiment. In all of the experiments some hemolysis occurred within one hour in suspensions containing 0.375 mg of gramicidin. This was true even in the presence of glucose. In each experiment, however, tyrothricin and tyrocidine were found to cause a greater degree of hemolysis than gramicidin. Hemolysis was observed to occur especially during the first 5 hours of incubation and only slight increases were noted thereafter.

When the cells were suspended in 5% glucose in distilled water, hemolysis was usually somewhat more marked. It must be pointed out, however, that this solution is hypotonic and the controls showed some hemolysis after 24 hours' incubation. In 10% glucose in distilled water gramicidin caused the same degree of hemolysis as in saline alone. Tyrocidine, however, showed less laking of the red cells in 10% glucose than in the saline solution. Saline suspensions containing either 5 or 10% added glucose caused some slight inhibition of the hemolytic effect. Similar results were obtained when rabbit's erythrocytes were used, but hemolysis always occurred with smaller concentration of the 3 substances.

Comment. The results obtained in these studies agree with Dubos[5] in that the marked hemolytic effect of tyrothricin seems to be due chiefly to its tyrocidine content. In some experiments we have observed hemolysis of rabbit's erythrocytes with as little as 0.000005 mg of tyrocidine. Gramicidin, while not nearly as hemolytic as tyrothricin or tyrocidine, has been found to lake red cells. This hemolytic action is not inhibited in 5 or 10% glucose in distilled water, and is only slightly inhibited in 5 or 10% glucose in

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† The tyrothricin was obtained from Lederle Laboratories, Inc., and Sharp and Dohme, Inc. Gramicidin was obtained from Sharp and Dohme, Inc., and was also made in our laboratories.

TABLE I.
Hemolytic Effect of Gramicidin on Human Red Cells.

	0.85% saline						5% glucose				5% glucose in saline			
	Tyrothricin		Gramicidin		Tyrocidine		Gramicidin		Tyrocidine		Gramicidin		Tyrocidine	
Mg	1°	24°	1°	24°	1°	24°	1°	24°	1°	24°	1°	24°	1°	24°
3.12			++	+++			++	+++			++	+++		
.75	++	+++	+	+++	++	+++	++	+++	+++	+++	+	++	++	+++
.185	++	+++	0	++	++	+++	+	+++	++	+++	0	++	++	+++
.09	+	+++	0	++	++	+++	+	+++	++	+++	0	+	++	+++
.045	+	+++	0	+	+	+++	0	+++	+	+++	0	0	+	+++
.022	+	+++	0	+	+	+++	0	++	+	+++	0	0	0	++
.011	0	++	0	+	+	++	0	++	+	+++	0	0	0	++
.005	0	++	0	0	0	++	0	++	0	+++	0	0	0	+
.0025	0	+	0	0	0	+	0	+	0	++	0	0	0	+
.0012	0	+	0	0	0	+	0	0	0	++	0	0	0	0
.0006	0	0	0	0	0	0	0	0	0	++	0	0	0	0
.0003	0	0	0	0	0	0	0	0	0	++	0	0	0	0
.00015	0	0	0	0	0	0	0	0	0	+	0	0	0	0
.00007	0	0	0	0	0	0	0	0	0	+	0	0	0	0

+++	Complete hemolysis.
++	Intermediate hemolysis.
+	Trace of hemolysis.

Addendum: The above experiments were repeated using gramicidin obtained through the courtesy of Drs. Dubos and Hotchkiss. Hemolysis was slight and delayed in all solutions; it was not completely prevented by the addition of glucose. The inhibition was most marked in 10% glucose in distilled water, the degree of inhibition being about 500-fold.

0.85% saline. Since the addition of either 5 or 10% glucose causes the same degree of inhibition, the amount of added glucose apparently is not an important factor.

Conclusions. Tyrothricin is markedly hemolytic for human and rabbit's erythrocytes. The hemolytic activity seems to reside primarily in the tyrocidine fraction, although gramicidin also exhibits a definite hemolytic effect. The addition of glucose to saline solution causes only slight inhibition of the hemolytic action.

13274 P

Peripheral Vascular Responses to General Anoxia.*

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From the May Institute for Medical Research, The Jewish Hospital, Cincinnati, Ohio.

In view of the current interest in the problem of general anoxia in man, it was considered important to study the local changes in blood flow in the extremities consequent to the inhalation of an oxygen-poor gas mixture.

The investigation was performed upon 9 normal, adult subjects, using the venous occlusion plethysmograph method. Blood flow determinations, expressed in cc per minute per 100 cc limb volume, were made upon the hand, leg, and forearm, according to the technic previously described.[1] Ten to 15 control readings were first obtained with an anesthesia mask firmly fitted to the face, but with the subject breathing air from the room. Then a gas mixture of approximately 10% oxygen and 90% nitrogen was allowed to enter the mask at atmospheric pressure for periods of 10 to 20 minutes; the exhaled air being automatically discharged into the room through an outlet tube and flutter valve. Blood flow determinations were made every 2 minutes during this interval. At the completion of the period of relative anoxia, another set of control readings was obtained with the mask still in place, but with the subject again breathing room air. The pulse rate, blood pressure, and respiration were recorded simultaneously with the blood flow readings.

The hand was examined in 4 subjects and in each instance there

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* Aided by the Samuel and Regina Kuhn Fund.

[1] Abramson, D. I., Zazeela, H., and Marrus, J., *Am. Heart J.*, 1939, **17**, 194, 206; Ferris, E. B., Jr., and Abramson, D. I., *Am. Heart J.*, 1940, **19**, 233.

was an almost immediate decrease in blood flow coincident with the onset of the period of anoxia, and a return to, or even beyond, the control level within a few minutes after atmospheric air was inspired. In 2 of these instances, however, a definite diminution in hand blood flow had been observed with the application of the mask alone and before the oxygen-poor mixture was breathed; the vasoconstrictor response being further accentuated by the latter procedure.

The leg was examined in 9 subjects and in most instances no significant changes in circulation were noted during the first 5 minutes of anoxia. In the next 10 or 15 minutes, the rate of blood flow began to rise, to attain an average maximal increase of 30% above the control readings. This augmented flow was maintained during the remainder of the period, but quickly returned to the previous level with the readmission of room air into the anesthesia mask (Fig. 1). In the 6 instances in which the forearm was examined, the response was similar to that observed in the leg.

The pulse rate began to rise almost immediately after the onset of the period of anoxia (Fig. 1), to attain an average maximum increase of 23 beats per minute. As soon as this period was terminated, the pulse rate quickly dropped, in some instances tempo-

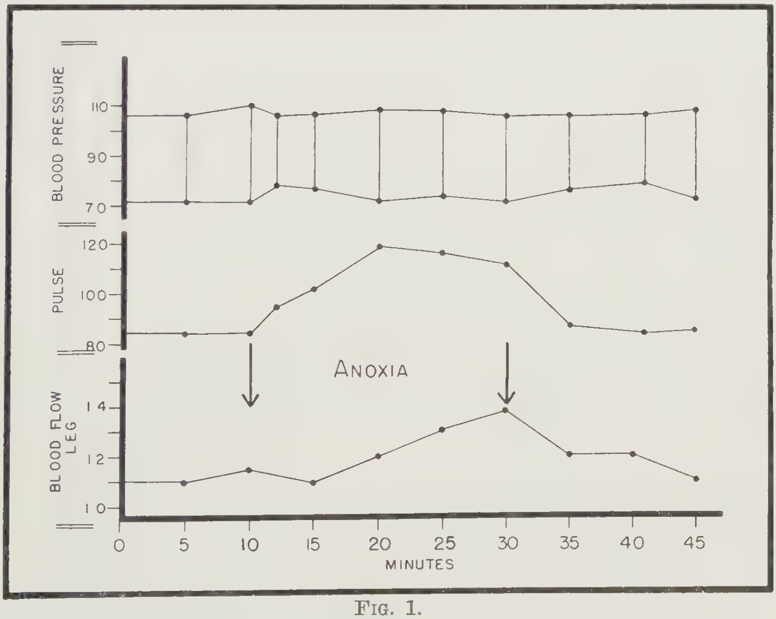

Fig. 1.

Effect of inhalation of 10.15 % oxygen mixture for period of 20 minutes (as indicated by interval between arrows). Blood flow in cc per min per 100 cc limb vol.

rarily reaching a lower level than that observed at the beginning of the experiment. The blood pressure for the most part showed no significant alteration during the period of anoxia. The respiration either increased an average of 4 per minute, or the respiratory movements were deepened without any alteration in rate.

From the foregoing, it appears that the inhalation of a 10% oxygen mixture, equivalent to an altitude of 20,000 feet, produced a moderate increase in the rate of resting peripheral blood flow. In respect to the hand, it is quite possible that the almost immediate decrease in flow was due to the apprehension associated with the procedure rather than to the anoxia *per se*, for it has been shown that the vessels in this region respond markedly to most psychic and noxious stimuli.[2] The observation that no change in blood flow was noted in the leg and forearm during the first 5 minutes of anoxia is readily understandable, since that period of time was probably required for the oxygen level in the blood to reach the new equilibrium with the oxygen in the mixture entering the mask. The fact that a greater than 50% decrease in oxygen content (from 20.9% in air to 10% in the mixture) produced only a moderate change in peripheral blood flow can also be easily explained, if one considers that in the normal subject under resting conditions, only a small proportion of the total quantity of oxygen in arterial blood is removed in passage through the muscle vessels. It would be expected, therefore, that during a state of moderate anoxia there would be no necessity for any marked increase in the rate of peripheral blood flow in order to satisfy the oxygen requirements of the resting tissues.

Summary. Inhalation of a mixture of 10% oxygen and 90% nitrogen produced a moderate increase in blood flow through the leg and forearm and a decrease through the hand. These changes in peripheral blood flow were associated with a significant augmentation in pulse rate, a slight increase in the amplitude or number of respirations and no definite change in blood pressure.

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[2] Capps, R. B., *J. Clin. Invest.*, 1936, **15**, 229; Kunkel, P., Stead, E. A., Jr., and Weiss, S., *J. Clin. Invest.*, 1939, **18**, 225; Abramson, D. I., and Ferris, E. B., Jr., *Am. Heart J.*, 1940, **19**, 541.

13275 P

A Hemophilia-Like Disease in Swine.*

A. G. Hogan, M. E. Muhrer and R. Bogart.

From the Laboratory of Agricultural Chemistry, University of Missouri, Columbia, Mo.

During the last few years a number of swine in the Missouri Agricultural Experiment Station† herd have died from hemorrhage, either spontaneous or as a result of minor wounds. All of the affected animals are closely related. It is evident that the blood-clotting mechanism is seriously defective, and a study was initiated in an attempt to classify the abnormality.

Bleeding Time. If determined by Duke's method[1] the bleeding time is within the normal range. As shown in Table I though, if an incision of any extent is made the bleeding time is prolonged indefinitely.

Coagulation Time. The procedure followed is essentially the same as the Lee-White[2] modification of Howell's method. The blood sample is taken from an ear vein, collected in cold paraffin coated tubes, and placed in an ice bath. The results reported in Table I show that the coagulation time was 5 times that of normal blood. The blood clots slowly, but after it forms it retracts in normal time.

According to the prevailing theory at least 4 components of the blood are intimately concerned with blood coagulation: (1) calcium; (2) prothrombin; (3) fibrinogen; (4) thromboplastin, which presumably is released from the platelets and other formed elements. Each one has been subjected to preliminary investigation.

TABLE I.
Bleeding Time, and Coagulation Time of Blood.

Observation	Type of animal	
	Normal	Defective
Bleeding time, sec.		
1 cm stab in ear with 18-gauge needle	140	155
1 cm slit in edge of ear	215	bled to death
Coagulation time, min.	28	145

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* Contribution from the Departments of Agricultural Chemistry and Animal Husbandry, Missouri Agricultural Experiment Station. Aided by a grant from the John and Mary R. Markle Foundation. Journal Series No. 761.

† A coöperative project with the Regional Swine Breeding Laboratory of the Bureau of Animal Industry, U. S. D. A.

[1] Duke, W. W., *J. A. M. A.*, 1910, **55**, 1185.

[2] Lee, R. I., and White, P. D., *Am. J. M. Sci.*, 1913, **145**, 495.

Calcium Time. According to our observations the calcium time[3] is not less than the coagulation time, and the serum calcium was shown by analysis to be well within the normal range. Our data on calcium are omitted from the tabulations.

Fibrinogen Content. Oxalated plasma was diluted with isotonic saline and recalcified. The resulting fibrin was removed and determined quantitatively. The data shown in Table II indicate that the blood of defective animals contains slightly less fibrinogen than does that of normal controls, but the quantity is more than enough to form a normal clot.

Prothrombin Time and Thromboplastin. The prothrombin time was determined both by the method of Howell[4] and of Smith, *et al.*[5] The prolonged prothrombin time obtained by Howell's method could be due to an abnormally low level of either prothrombin or thromboplastin. Since the prothrombin time by Smith's method is normal, it is concluded that the blood of the defective animals is deficient in available thromboplastin. The data are summarized in Table II.

TABLE II.
Quantitative Determinations Related to the Coagulation Time of Blood.

Type of animal	Prothrombin time		Fibrinogen in plasma %	Platelets	
	Howell min	Smith sec		No. per mm³ × 1000	Vol. %
Normal	15	30	.54	205.0	.42
Defective	110	30	.45	179.7	.59

Blood Platelets. The platelet counts were made by the indirect method, essentially as described by Gradwohl[6] and by the direct method of Rees and Ecker.[7] Platelet volume was also determined by the method of Van Allen.[8] The data in Table II indicate that the platelet count in defective swine is not significantly different from that in normal animals. However, the volume of platelets is higher in the blood of defective animals, presumably because they are more stable. Platelet counts were made on blood that had stood for varying periods of time, following the procedure of Howell and Cekada.[9] The blood was collected in paraffin-coated tubes which were kept cool in an ice bath. At regular intervals of time blood smears were made on a glass slide, dried, and stained by Wright's method.[10] The specimens were examined for the relative number of platelets and for the degree of agglutination that had occurred. The data presented in Table III show that the platelets disintegrate more rapidly in normal than in abnormal blood. These studies indicate that the platelets from defective animals are abnormally stable, and at present this is the most obvious explanation of the abnormalities observed.

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[3] Todd, J. C., and Sanford, A. H., *Clinical Diagnosis by Laboratory Methods*, 8th Ed., Saunders, Philadelphia, 1936, p. 217.

[4] Howell, W. H., *Arch. Int. Med.*, 1914, **13**, 76.

[5] Smith, H. P., Ziffren, S. E., Owen, C. A., and Hoffman, G. R., *J. A. M. A.*, 1939, **113**, 380.

[6] Gradwohl, R. B. H., *Clinical Laboratory Methods and Diagnosis*, Mosby, St. Louis, 1935, p. 258.

[7] Rees, H. M., and Ecker, E. E., *J. A. M. A.*, 1923, **80**, 621.

[8] Van Allen, C. M., *J. Lab. Clin. Med.*, 1926, **12**, 282.

The symptoms resemble hemophilia more closely than they do any other disease that has come to our attention.

TABLE III.
Stability of Platelets.

Type of animal*	Min. after collection	No. platelets per 1000 erythrocytes	Remarks
Normal	5	39	A considerable number adhering in pairs
Defective	5	32	No agglutination
Normal	30	17	Agglutinated and ragged
Defective	30	27	No agglutination

*The data are averages of 2 normal and of 2 defective animals.

13276

Return of Hepatic Vitamin A in Rats after Depletion by Methylcholanthrene.

T. U. MARRON. (Introduced by H. A. Mattill.)

From the Division of Biochemistry, State University of Iowa, Iowa City, and the Department of Pathology, Iowa Lutheran Hospital, Des Moines, Iowa.

Goerner[1] demonstrated that a gelatin suspension of 1,2,5,6-dibenzanthracene injected intraperitoneally into rabbits markedly decreased the vitamin A content of the liver mitochondria. In surviving animals the vitamin content of these structures was slowly restored after the drug was discontinued. Later Goerner and

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[9] Howell, W. H., and Cekada, E. B., *Am. J. Physiol.*, 1926, **78**, 500.

[10] Wright, J. H., *J. A. M. A.*, 1910, **55**, 1979.

[1] Goerner, A., *J. Biol. Chem.*, 1937, **122**, 529.

Goerner,[2] in examining livers from dibenzanthracene-treated rabbits and rats, found decreases in hepatic vitamin A that ranged to zero.

At the time the latter paper appeared we had completed analogous experiments on the Sprague-Dawley strain of white rats. Twenty-five animals of both sexes and of various ages were given intraperitoneal injections of methylcholanthrene (Eastman) in lard filtered through a medium paper at 37°C. Doses of 6 mg or less were given over a period of 3 weeks; some animals received more frequent doses than others. For every methylcholanthrene animal another was similarly injected with phenanthrene in 37° lard to find the effect of that non-carcinogenic hydrocarbon. Controls for both groups were animals injected with the lard alone. Animals were sacrificed at periods of one to 20 days after the last injection, and the excised whole livers were digested and extracted by a modification of the method of Davies.[3] Vitamin A was determined, excluding carotene, by a modified Carr-Price reaction.[4, 5] All of the rats that had received 22 to 54 mg of methylcholanthrene and had been allowed to live 22 days or more showed very little or no hepatic vitamin A. The average liver weight, expressed as percent of body weight, was greater than that of the controls or stock animals, but not abnormal according to Donaldson.[6] The livers of phenanthrene and lard treated animals compared well in vitamin A content and size with stock animals examined.

These animals had not been allowed to live long enough after the drug administration had been discontinued to demonstrate any recovery effect similar to that mentioned by Goerner[1] for rabbits that had received dibenzanthracene. Nor did Goerner and Goerner[2, 7] allow dibenzanthracene-treated rats to survive so that studies of the hepatic vitamin A could be made in the recovery period. Therefore, 20 white rats of an unknown, inbred strain, weighing 100 to 200 g, were each given 2 intraperitoneal injections of 6 mg methylcholanthrene in 37° lard 30 days apart. About 75 days after the last injection normal weight gains were observed; and 10 days later half of the animals with an equal number of lard controls of both sexes were sacrificed. Fluorescence microscopy[8] was substituted for a chemical method of detecting vitamin A. The methylcholanthrene-treated animals were found to have hepatic vitamin stores equal to those of the lard controls and stock animals. After 9 months the remaining animals were still normal as to both vitamin A stores and liver histology.

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[2] Goerner, A., and Goerner, M. M., *Am. J. Cancer,* 1939, **37**, 518.

[3] Davies, A. W., *Biochem. J.*, 1933, **27**, 1770.

[4] Rosenthal, E., and Erdelyi, J., *Biochem. Z.*, 1933, **267**, 119.

[5] Rosenthal, E., and Wiltner, M., *Biochem. J.*, 1935, **29**, 1036.

[6] Donaldson, H. H., *The Rat,* Memoirs of the Wistar Institute, No. 6, 1924.

[7] Goerner, A., and Goerner, M. M., *J. Nutr.*, 1939, **18**, 441.

[8] Popper, Hans, PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 133.

The purity of the hydrocarbon used was confirmed by the manufacturer, and a new rechecked lot was furnished, with which the experiments were repeated on 10 animals with 5 lard controls. The dosage was 20 mg methylcholanthrene divided into 3 portions given during 30 days. Weight gains were resumed about 90 days after the first injection. At 120 days the livers of the 15 animals were alike as to normal stores of vitamin A[8] and absence of liver pathology; complete blood counts were normal as established for the colony.

All of the animals in the 3 experiments were on a stock diet of Purina Dog Chow. No exceptional loss of appetite was ever noticed. No tumors ever appeared; and no animals died except of internal injury at injection.

Conclusion. Methylcholanthrene will deplete rat livers of vitamin A in 3 to 6 weeks; phenanthrene or lard injections will not. Some strains of white rats are not readily susceptible to tumor production by intraperitoneal injections of the drug and regain their ability, after discontinuance of injections, to store vitamin A in the liver.

I express indebtedness for assistance from Drs. J. S. Weingart, C. Carruthers, I. B. Johns, H. W. Beams, and technical aid from I. Carlson and I. W. Carlson.

13277

Non-Effect of Ovariectomy on the Twenty-fifth Day of Pregnancy in the Rhesus Monkey.

Carl G. Hartman. (Introduced by George W. Corner.)

From the Carnegie Laboratory of Embryology, Baltimore.

It was demonstrated[1] that the pregnant Rhesus monkey is able to carry its fetus to term despite surgical removal of the ovaries. Five cases were cited which were castrated on days 95, 89, 46, 35, 31 of gestation, respectively. To these it is now possible to add another and more crucial case castrated 25 days after ovulation and fertilization.

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[1] Hartman, Carl G., *Anat. J. Obst. and Gynec.*, 1939, **37**, 287.

The twenty-fifth day was selected for this experiment because of the following theoretical considerations. First and foremost is the fact that in the monkey at this period the corpus luteum involutes in every normal pregnancy, assuming a smaller volume and more compact internal structure characteristic of the greater part of pregnancy.[2] At this time, too, the definitive vascular relationship between mother and fetus is fairly well established, the destruction of maternal tissue by the invading trophoblast has nearly ceased, and the chorionic hormone is about to disappear from the urine[3] and the blood.[4] Finally, the pregnant female, from this point on, gradually loses all sex interest, in which behavioral state she remains throughout pregnancy.[5]

The new case concerns monkey No. 637 of the Carnegie colony, which was received on May 19, 1938, weighing 4010 g. At the time of ovariectomy she weighed 8 kilos. The recorded menstrual cycles were 28, 28, 26, 35, 26, 29, and 22 days in length, all ovulatory. She was hysterotomized twice: On January 16, 1939, an 11½-day embryo was recovered and the right ovary removed; on January 21, 1940, a similar embryo was expected but not found in the excised endometrium.

The next ovulation, mating and conception occurred on November 21, 1940, day 10 of the cycle. On December 6 the placental bleeding first manifested itself. On December 16, the twenty-fifth day of pregnancy, the surviving ovary and fallopian tube, together with as much of the ovarian mesentery as possible, were removed and it was made certain that no ovarian tissue was left behind. The ovary was serially sectioned and seen to contain the typical corpus luteum of this stage.

By rectal bimanual palpation the course of pregnancy was followed and found to proceed normally except for a considerable blanching of the sex skin in the second week after castration and the appearance of a few erythrocytes on January 2. No "therapeutic" treatment, in view of this "threatened abortion," was, however, instituted. The sex color gradually returned to the brilliant hue of pregnancy, which persisted to term. A 440 g female baby (about average weight for the Rhesus monkey) was born on May 5, 1941, after a gestation period of 165 days, the exact average for the Rhesus monkey. The postpartum involution of the uterus proceeded as in unoperated cases and the baby nursed and developed according to expectation up to the time of this report (July, 1941).

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[2] Corner, G. W., Bartelmez, G. W., and Hartman, C. G., *Anat. Rec.*, 1936, **59**, 433.

[3] Hamlett, G. W. D., *Am. J. Physiol.*, 1937, **118**, 664.

[4] Delfs, E., *Anat. Rec.*, 1941, **79** (Suppl. 2), 17.

[5] Ball, J., *Anat. Rec.*, 1937, **67**, 507.

Strangely enough, nursing, like pregnancy, proved an efficient stimulus for the maintenance of bright red color of the sex skin even in the absence of ovarian tissue. For about 2 weeks postpartum the color was distinctly less intense (6 on a scale of 10) but after that rose to 8 and 9, at which it was being maintained at the time of this report, 2 months after parturition. This raises the question: Whence comes the hormone conditioning the sex color? One thinks of the placenta as substituting for the ovary during pregnancy, but during lactation we must look elsewhere for the source of "ovarian" hormones. As a matter of fact the monkey sex skin may be experimentally reddened by all the estrogens, by androgens, by desoxycorticosterone[6] and even by progesterone[7] alone. It is quite possible that adrenotropic hormone of the hypophysis is involved, stimulated in pregnancy by placental hormones, in lactation by the act of suckling. Other facts also point to extragonadal, synergistic factors in the maintenance of the sex color of the Rhesus monkey. This problem will be discussed in another place.

Summary. Theoretical considerations predicted and the experiment here recorded have proved that in the rhesus monkey the ovaries are not necessary from the twenty-fifth day of pregnancy on for normal gestation, parturition at term, postpartum involution of the uterus and lactation. Surprisingly, the bright red color of the sex skin was maintained, in the absence of the ovaries, not only during gestation but also during lactation.

13278

Absorption of Sulfathiazole from the Gastrointestinal Tract, Pleura, and Peritoneum of Dogs.

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From the Department of Pharmacology, Medical College of Virginia, Richmond.

The recent literature contains many reports describing the use of sulfonamide drugs locally, particularly in the treatment of traumatic and operative wounds. Since these drugs have also been used locally in such highly absorptive areas as the pleural and peritoneal cavities,[1-6] it appears important to establish experimentally the extents to which they are absorbed from these surfaces in order that safe doses may be approximated.

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[6] Speert, H., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 189.

[7] Hartman, Carl G., and Speert, H., in press.

Hudson, Smith, and Selbie[6] applied sulfanilamide to the peritoneum of rabbits and noted the time necessary for complete absorption. Keeley[7] injected a suspension of this drug into the peritoneal cavity of dogs and determined the sulfanilamide content of the blood.

Experiments from this laboratory on sulfanilamide,[8] and the present experiments on sulfathiazole, were designed to simulate the local use of these drugs after thoracic and abdominal surgery, and to compare the degree of absorption from the pleura and peritoneum with that from the gastrointestinal tract.

Experimental. Four dogs were used for each of the 3 methods of administration, the procedure being identical with that previously described.[8] Determinations for sulfathiazole were made on blood taken immediately before and at regular intervals after the drug administration. The method of analysis was that of Marshall and Litchfield,[9] using sulfathiazole as the standard. In all cases the dose was 100 mg per kg. For the oral administration the drug was suspended in water and given by stomach tube after withholding food for 14 hours. For the intrapleural and intraperitoneal administrations the dogs were etherized, operated on under sterile conditions, and the powdered drug deposited into the pleural space, or on the intestines beneath the greater omentum. The incisions were closed and the dogs allowed to recover.

Results. The accompanying graph presents the sulfathiazole values found in the blood after the oral, intrapleural, and intraperitoneal administration of the drug. Each point represents the average of the determinations on 4 dogs. Statistical analysis* of our data showed that for every point, except one, there was no significant

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[1] Herrell, W. E., and Brown, A. E., *Proc. Staff Meet. Mayo Clinic,* 1940, **15**, 611.

[2] Dees, J. G., *Mississippi Doctor,* 1940, **18**, 215.

[3] Barondes, R. deR., *Clin. Med. Surg.,* 1940, **47**, 292.

[4] Adams, H. D., *Lahey Clin. Bull.,* 1940, **1**, 13.

[5] Rosenburg, S., and Wall, N. M., *Surg. Gyn. Obs.,* 1941, **72**, 568.

[6] Hudson, R. V., Smith, R., and Selbie, F. R., *Lancet,* 1941, **1**, 438.

[7] Keeley, J. L., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 458.

[8] Haag, H. B., Spealman, C. R., and McCue, H. M., *Surgery,* in press.

[9] Marshall, E. K., Jr., and Litchfield, J. T., *Science,* 1938, **88**, 85.

* According to Fischer's method for small samples.[10]

[10] Baten, W. D., *Elementary Mathematical Statistics,* John Wiley and Sons, Inc., N. Y., 1938, p. 271.

difference in the blood levels of sulfathiazole after absorption from the gastrointestinal tract, pleura, and peritoneum. The exception is at the 2-hour point in comparing absorption by the oral and intrapleural routes. At this point the t-value (3.87 for 6 degrees of freedom) was somewhat elevated. This would seem to indicate at this one point a significantly higher blood level after pleural than after oral administration.

The peak blood concentrations occurred 2 to 3 hours after administration. These values of 4.24, 4.56, and 5.54 mg per 100 cc of blood for the oral, peritoneal, and pleural routes respectively, are somewhat lower than those obtained in similar experiments from this laboratory using the same dose of sulfanilamide.[8] In the latter experiments the peak values found (in the same order) were 7.1, 8.2, and 8.2 mg per 100 cc of blood. Goodwin and Findlay[11] also found the blood level of sulfathiazole lower than that of sulfanilamide after application to uninfected wounds on the hind legs of rabbits.

Summary. With one exception (as discussed) no significant difference was found in the absorption of sulfathiazole as judged

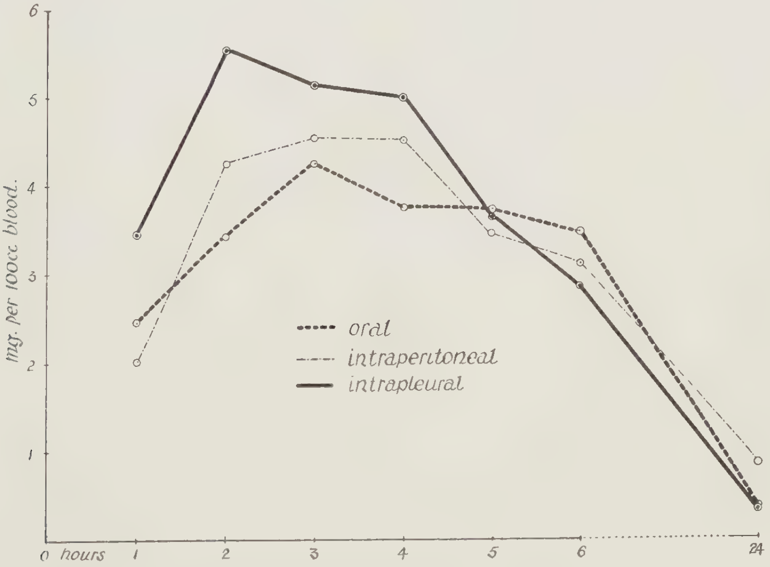

FIG. 1.
Comparative absorption rate of sulfathiazole in dogs following various routes of administration. Dose of sulfathiazole, 100 mg per kg.

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[11] Goodwin, L. G., and Findlay, G. M., *Lancet*, 1941, **1**, 691.

from studies of blood concentration, after oral, intrapleural, and intraperitoneal administration to dogs. Although these experiments are not directly comparable to the clinical use of sulfathiazole, they may well serve as a guide in the matter of dosage in the local application of the drug to the pleural and peritoneal cavities.

13279 P

Experimental Production of Tumors by Tars from Various Foods.

Y. Liu and C. H. Hu.

From the Department of Pathology, Peiping Union Medical College, Peiping, China.

In view of the successful production of tumors by crude coal tar[14] and its derivatives with known chemical composition,[1-4] interest has been directed to the question whether tars prepared from substances which we eat or smoke are also carcinogenic. Fukida and Kinosita[5] found that tar from rice bran is carcinogenic to the skin of mice. Kenneway[6] produced cancers and papillomas by human skin tar and yeast tar. Shibata[11] reported on the carcinogenic action of the tar from sugar. Roffo[8] found tumor growths in the stomach, liver, and cecum of rats fed on bread and milk to which was added animal fat or olive oil that had been heated to 350°C for half an hour. Widmark[13] succeeded in obtaining adenocarcinomas of breast with alcohol or petroleum-ether extract of roasted horse muscle, coffee and brown butter. Lü,[7] Taki,[12] and Sugiura,[10] etc., produced tumors on mice by tobacco tar. Roffo[9] was able to identify

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[1] Cook, J. W. Haslewood, G. A. D., Hewett, C. L., Hieger, I., Kenneway, E. L., and Mayneord, W. V., *Am. J. Cancer*, 1937, **29**, 219.

[2] Cook, J. W., and Kenneway, E. L., *Am. J. Cancer*, 1938, **33**, 50.

[3] Cook, J. W., and Kenneway, E. L., *Am. J. Cancer*, 1940, **39**, 381.

[4] Cook, J. W., *Nature*, 1940, **145**, 335.

[5] Fukida, T., and Kinosita, R., *Transact. Jap. Path. Soc.*, 1923, **13**, 636.

[6] Kenneway, E. L., *B. M. J.*, 1925, **2**, 1.

[7] Lü, Fu-hwa, *Frankf. Z. Path.*, 1934, **46**, 513.

[8] Roffo, A. H., *Bull. de l'Ass. franc. l'etude du cancer*, 1939, **28**, 556.

[9] Roffo, A. H., *Z. f. Krebsforsch.*, 1939, **49**, 588.

[10] Sugiura, K., *Am. J. Cancer*, 1940, **38**, 41.

[11] Shibata, S., *Higu, Kiyo.*, 1928, **12**, 298.

[12] Taki, S., *Osaka Igaku Z.*, 1937, **36**, 483.

[13] Widmark, E. M. P., *Nature*, 1939, **143**, 984.

[14] Yamagiwa, K., and Ichikawa, K., *Verhandl. d. jap. path. Gesell.*, 1915, **5**, 142.

the presence of 1-2 benzpyrene, a carcinogenic hydrocarbon, in tobacco tar.

The following experiment was carried out to find whether tars from certain common food articles also have carcinogenic properties.

Material and Method. The material consisted of polished rice, pea flour, soybean, casein, wheat gluten, whole wheat, corn, cabbage, pepper, beef, and pork. Each of these, in dry form, was placed in an iron retort to about one-half of its full capacity and was then heated on a strong gas flame for destructive distillation. The distillate was collected in a water-cooled receiver. To remove the small amount of water present, the distillate was shaken with an equal part of chloroform. The water fraction and the chloroform fraction were then separated. The chloroform was removed by distillation and the water fraction was concentrated on a steam bath. These two fractions were finally mixed together and used for the experiment.

For the purpose of painting, 11 groups of white mice of 10 each were used, one group for each kind of tar. The painting was done on alternate days. The area of the skin painted was about 1.5 cm square on the back at the level of the scapulae, the hair having been previously removed by a thin paste of barium sulphite, 1 part, and talcum powder, 3 parts. The animals were from our own stock, 3-4 months old, and weighing 20-25 g. When one animal died it was replaced by a new animal, so that the number of living animals in each group was kept constantly at 10.

For control, 5 animals were painted with coal tar which had been found to be carcinogenic.

Results. Up to the time of this report, 230 mice were painted for 15 to 343 days (animals dying in the first 2 weeks of the experiment were excluded). Of these, 4 developed papillomas (wheat gluten, 1; pepper, 1; cabbage, 2) in 52 to 315 experimental days. The 5 controls all developed tumors (3 papillomas and 2 carcinomas) in 60 to 83 days.

13280 P

Production and Apparent Prevention of a Dietary Liver Cirrhosis in Rats.

Floyd S. Daft, W. H. Sebrell and R. D. Lillie. (Introduced by Charles Armstrong.)

From the Divisions of Chemotherapy and Pathology, National Institute of Health, U. S. Public Health Service, Bethesda, Md.

Lillie, Daft and Sebrell,[1] among others in the past few years, have reported liver cirrhosis in animals given special diets. György and Goldblatt[2] reported liver injury as a regular complication in rats on a diet containing 10% casein and 22% fat. Necrosis or cirrhosis or both was observed from the 100th to 150th experimental days. They state "Addition of from 10 to 20 mg of choline daily reduced the incidence and severity of the liver injury but not to a great extent." In a later paper György, Poling, and Goldblatt[3] refer to this work and state "These changes can be prevented to a large extent by the addition of casein to the diet, but this is accomplished more effectively by the combined oral administration of cystine and choline." Blumberg and McCollum[4] reported that liver cirrhosis in rats on a diet containing 10% casein and 55 or 70% fat, occurring in 125 to 150 days on the lower fat intake and in 100 to 140 days on the higher, could be prevented by including 10 mg per gram of choline in the diet and could be slowed but not prevented by methionine at 25 mg per rat per day. Earle and Victor[5] reported liver cirrhosis in 14 to 27 days in rats given a diet containing 10% cystine. They also reported cirrhosis in one of a number of rats given a diet containing 5% cystine.

We wish to report at this time the consistent and fairly rapid production of liver cirrhosis in rats on a low protein, low fat diet with added cystine and the apparent prevention of the cirrhosis by choline, methionine and casein, singly or in combination.

Rats were placed at weaning or shortly thereafter on our diet No. 545. consisting of leached casein 4%, cystine 0.5%, cod liver oil 2%, Wesson Oil 3%, Osborne and Mendel salt mixture 4%, and cornstarch 86.5%. They were given approximately 20% alcohol in place of drinking water and daily supplements of 100 μg of thiamin, 50 of riboflavin, 20 of pyridoxine, 50 of calcium pantothenate and 1 mg of nicotinic acid. Of 20 rats examined to date, all died between the 46th and 111th experimental days (average 83) and all showed moderate to marked liver cirrhosis on gross and histological examination. In a preventive experiment, 40 litter mates were given 20 mg of choline daily in the supplement or 0.7% of methionine in the diet or the dietary casein was raised from 4% to 30% at the expense of the cornstarch. Combinations of these changes were also employed. None of the rats sacrificed at the end of 90 or 140 experimental days or dying during the experiment showed any evidence of liver cirrhosis. Most of them are alive and apparently healthy, some after an experimental period as long as 6 months. The experiment is being continued to make certain that this represents a true prevention rather than a slowing of the cirrhotic process.

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[1] Lillie, R. D., Daft, F. S., and Sebrell, W. H., *Pub. Health Rep.*, 1941, **56**, 1255.

[2] György, P., and Goldblatt, H., Proc. Soc. Exp. Biol. and Med., 1941, **46**, 492.

[3] György, P., Poling, E. C., and Goldblatt, H., Proc. Soc. Exp. Biol. and Med., 1941, **47**, 41.

[4] Blumberg, H., and McCollum, E. V., *Science*, 1941, **93**, 598.

[5] Earle, D. P., and Victor, J., *J. Exp. Med.*, 1941, **73**, 161.

Since rats given diet number 545 and water but no alcohol have died with an indistinguishable liver pathology, it appears that the diet is here the essential factor.

13281

Production of Cytoplasmic Inclusions in Liver Cells of Rats Injected with Certain Proteins.

JULIA T. WELD, WILLIAM C. VON GLAHN AND LUCY C. MITCHELL.
From the Department of Pathology, College of Physicians and Surgeons, New York City.

In connection with studies on the effect of intravenous injection of rats with hemolytic streptococcus extract toxin[1] * (toxic serum extracts of hemolytic streptococci), we discovered that under certain conditions large cytoplasmic bodies appear within the liver cells. On further study, we found that other proteins also caused the development of similar bodies. This preliminary paper deals with the methods used for the production of this lesion and its description and is based on a study of 850 rats.

In Table I are presented: first, the substances that cause the lesion when injected intravenously; second, the percentage of body weight of the substance injected; third, the time elapsing between injection and autopsy. In Table II are presented the substances studied that, under the conditions of these experiments, have not produced inclusions.

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[1] Weld, J. T., *J. Exp. Med.*, 1934, **59**, 83.

* In this work the streptococcus extract toxin is referred to as "streptococcus toxin."

The important factors in the production of the lesion are as follows:

Age of Animal. In order to obtain lesions regularly, rats should be at least 7 weeks old. When younger rats are used, the results are often negative.

TABLE I.
Proteins Producing Liver Inclusions when Injected Intravenously into Rats.

	Substances injected intravenously	% of body weight injected	Autopsy—Hour after injection
1	Streptococcus toxin (1) prepared with either horse serum or rat serum	1	2,4,5,18,24,48 or 56 hr
2	Normal sheep serum	1,2 or 3	Immediately, 10 or 30 min., 2,4,5,18, 24,48,56 hr
3	Sheep serum albumen or sheep serum globulin	2	4 hr
4	Normal horse serum into specifically sensitized rats (anaphylactic shock) (2)	1.5	3,7,15,25,46,48,56 min. 1,2,3,4,5,6,18,24,48 hr
5	Fresh egg albumen	1	4 hr
6	Crystallized egg albumen combined to R.-salt dye (3)*	1 or 1.5	4 hr
7	Sheep serum albumen combined to R.-salt dye*	1.5	4 hr

*We are indebted to Dr. H. Smetana for this preparation.

TABLE II.
Proteins That Have Not Produced Liver Inclusions when Injected Intravenously into Rats.

	Substance injected intravenously	% of body weight injected	Autopsy—Hour after injection
1	Normal horse serum	1, 2 or 3	2,4,5,18,24 hr
2	Normal rat serum	2	4 hr
3	Streptococcus toxin* (1) heated to 56° for one hour	2	4 hr
4	Horse serum extract of non-hemolytic streptococcus	3	4 hr

*Extract is non-hemolytic and non-toxic.

Amount of Substances Injected. In general, the number and size of the inclusions obtained appear to be directly proportionate to the percentage of body weight of inoculum. To produce numerous and large inclusions, 1 to 3% of the body weight of the substance should be injected.

Earliest Time After Injection of Appearance of the Inclusions. The time of the appearance of the inclusion after injection varies with the material injected. Thus, when streptococcus toxin was injected, the inclusions first appeared at 2 hours after injection but were absent in 30 minutes. (No rats were killed sooner after injection than this.)[†] With sheep serum (2% of body weight) numerous "early" inclusions were found in 9 of 10 rats within 10 minutes after injection and even in one rat killed immediately. In the anaphylactic rats[2] inclusions were usually present 1 hour after injection and in several rats in less than 1 hour. With the other substances used, we have data only on rats killed 4 hours or more after injection (Table I).

Progress of the Lesion. The bodies appear to be most conspicuous and well-defined when the rats are killed 4 to 5 hours after injection. After 18 to 24 hours they are fewer and less well outlined. At 48 hours they are scarce and stain faintly. Merely a few pale faded globules were found in one only of 5 rats killed 56 hours after the injection of 2% of sheep serum.

Effect of Dietary Deficiency on the Production of the Liver

TABLE III.
Staining Reactions of Inclusions in Hepatic Cells.

Stains	Early. Granular stage After injection of a. sheep serum; b. horse serum into specifically sensitized rats	Late. Homogeneous stage After injection of a. sheep serum; b. horse serum into specifically sensitized rats	Late. Homogeneous stage After injection of streptococcus toxin
Hematoxylin and Eosin	Pale red	Yellow occasionally red	Metallic red or yellow
Azan Carmine	Blue	Brilliant orange	Brilliant orange
Phosphotungstic Acid Hematoxylin	Yellow	Blue	Blue*
Giemsa	Pale red or purplish red	Yellow or purplish red	Red

*Some inclusions are yellow or muddy yellow, probably early stage.

[†] In this work all rats were killed with ether.

[2] Weld, J. T., and Mitchell, L. C., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 168.

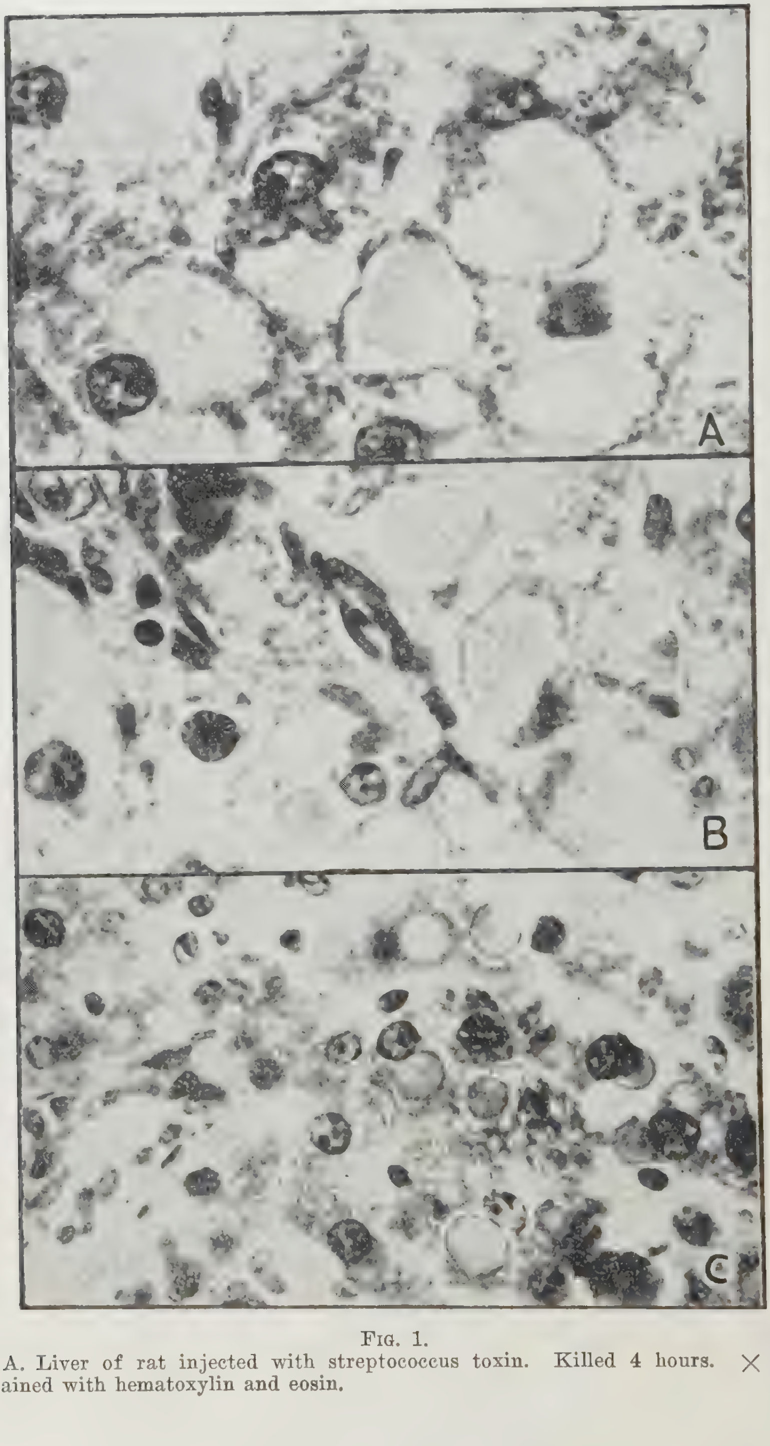

Fig. 1.

A. Liver of rat injected with streptococcus toxin. Killed 4 hours. × 500. Stained with hematoxylin and eosin.

B. Liver of rat sensitized to horse serum and reinjected with horse serum 13 days later (anaphylactic shock). Died 15 min. × 1050. Stained with hematoxylin and eosin.

C. Liver of rat sensitized to horse serum and reinjected with horse serum 13 days later (anaphylactic shock). Died 25 min. ×1050. Stained with hematoxylin and eosin.

Lesion. Except in the case of streptococcus toxin, no definite influence of dietary deficiency (essential amino acids, vitamin B complex or minerals) upon the formation of the bodies could be detected. Dietary deficiencies did seem to favor the development of the inclusions after injection of the streptococcus toxin.

Symptoms. All the substances that produce the liver lesions cause definite prostration lasting varying periods of time; and there appears to be a rough correspondence between the severity of the symptoms and the number of inclusion bodies. However, the bodies may persist after recovery from the prostration.

Description of the Inclusion Bodies. The cytoplasmic masses appear first as agglomerations of granules with fibrin within the liver cells (Fig. 1 A and B) that soon fuse into a roughly spherical or oblong homogeneous body (Fig. 1 C). This lies in a clear vacuole and often pushes the nucleus to one side. In unstained preparations they are greenish and have the same refractivity as erythrocytes. Their reaction to various stains is shown in Table III. Bodies of similar appearance are found also within the Kupffer cells, but they appear only after 2 hours and are smaller in size. Following injection of R-salt-protein,[3] the liver cell inclusions show a varying intensity of red staining; the Kupffer cells are filled with bright red particles. The inclusions do not stain as fat or glycogen.

The interesting problem, that we are studying further, is the chemical relation of these bodies in the liver to the injected protein.

13282

Demonstration of Myelolytic Substances in Disseminated Sclerosis.

Arthur Weil and Gert Heilbrunn.

From the Institute of Neurology, Northwestern University, and the Elgin State Hospital.

Following the study of the myelolytic action of substances like saponin, bile salts and snake venoms upon the myelin sheaths of rat spinal cords in test tube experiments,[1, 2] the attempt was made to demonstrate the presence of myelolytic substances in disease processes which are characterized pathologically by a selective destruction of myelin in the central nervous system. The classical disease which lends itself best for such a study is disseminated sclerosis, and many authors before had already assumed *a priori* the presence of myelolytic substances in this disease without being able to demonstrate them with certainty.[3]

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[3] Heidelberger, Michael, and Kendall, Forrest E., *J. Exp. Med.*, 1933, **58**, 137.

Weil, Luhan and Balser[4] demonstrated in the urine of about 70% of cases of disseminated sclerosis the presence of substances which acted in test-tube experiments upon fresh rat spinal cord in a similar way as sodium taurocholate. These substances were not identical with the lipolytic ferments which Brickner claimed to have demonstrated in this disease,[5] but the presence of which subsequently could not be confirmed by Richards and Wolff.[6] We did not find myelolytic substances in normal urines or in urines of patients suffering from syphilis of the central nervous system, from subacute combined degeneration or other degenerative diseases of the brain and spinal cord. They were present, however, in cases of post-encephalitic Parkinsonism and in certain diseases of the liver. It appeared that the amount of such substances present in cases of disseminated sclerosis varied with the periods of remissions and exacerbations and that a positive reaction was more frequently found during periods of exacerbations. Since then such experiments have been continued and at present the results are as shown in Table I.

TABLE I.
Myelolytic Substances Present in Urines.

Diagnosis	No. of patients Total	 +	 —	% of total +	 —
Disseminated sclerosis	165	115	50	70	30
General paresis	29	0	29	0	100
Post enc. Parkinsonism	43	28	15	65	35
Other dis. of c.n.s.	83	4*	79	5	95
Normal controls	25	0	25	0	100

*One case each diagnosed as Huntington's chorea, Wilson's disease, *Dystrophia myotonica* and amyotrophic lateral sclerosis. Nine cases of disseminated sclerosis which had given a positive reaction were verified by autopsy.

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[1] Weil, A., *Arch. Path.*, 1930, **9**, 828.

[2] Weil, A., *Arch. Exp. Path. u. Pharm.*, 1930, **154**, 228.

[3] Weil, A., *J. A. M. A.*, 1931, **97**, 1587.

[4] Weil, A., Luhan, A. J., and Balser, B. H., *Transact. Am. Neur. Assn.*, 1935, **61**, 142.

[5] Brickner, R. M., *Arch. Neurol. and Psych.*, 1930, **23**, 715.

[6] Richards, C. H., and Wolff, H. C., *Arch. Neurol. and Psych.*, 1940, **43**, 59.

The original technic which had been used was the following: 200 cc of urine collected in sterilized bottles and which had been preserved in the icebox were evaporated at a temperature not exceeding 50°C *in vacuo*. The residue was redissolved in 5 cc of 0.85% NaCl solution and incubated with a small piece of fresh rat spinal cord which had been removed under aseptic precautions. Following incubation for 16 hours at 37°C the spinal cord was fixed in formaldehyde solution, embedded in paraffin, cut longitudinally (15 micron) and stained for myelin sheaths with the Weil method. In normal controls incubated with rat spinal cord the myelin sheaths are seen under the microscope as narrow dark black staining bands (Fig. A). But in spinal cords incubated with urines from active cases of disseminated sclerosis they appeared broken up into fine granules (Fig. C), resembling somewhat the reaction which is seen following incubation of spinal cords with solutions of sodium taurocholate (Fig. B), though the granulation is much finer after incubation with disseminated sclerosis urines. It becomes coarser and more distinct following incubation with acetone precipitates (Fig. D).

Bacterial contamination of the urines was avoided as much as possible by taking the precautions as described above. But even normal urines which had been kept intentionally at room temperatures for 2 days did not show the myelolytic action after incubation with rat

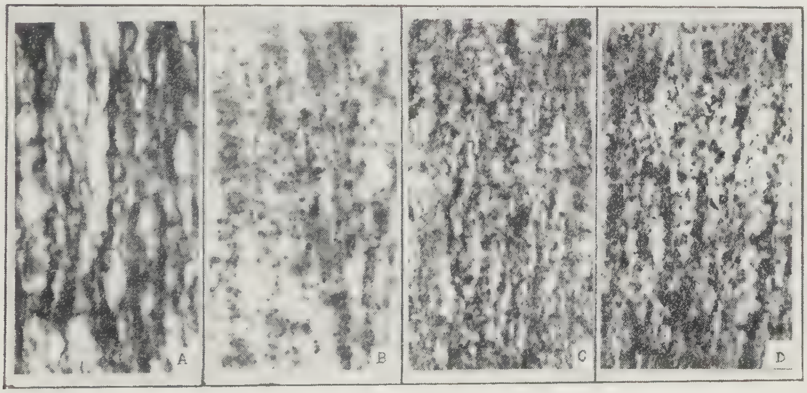

Fig. 1.

Spinal cords of rats after 16 hours' incubation at 37°C were fixed in formalin solution, embedded in paraffin; sections were stained for myelin sheaths with the Weil method. Magnification 910 ×. A. 200 cc of normal urine, concentrated to 5 cc. B. 30 mg of sodium taurocholate dissolved in 3 cc of water. C. 200 cc of urine from a patient suffering from disseminated sclerosis, concentrated to 5 cc. D. 700 mg of acetone precipitate from alcoholic extract of concentrated urine of a case of disseminated sclerosis, dissolved in 7 cc water. For description of the histologic changes compare text.

spinal cord. Active urines from cases of disseminated sclerosis after having been boiled for 5 minutes showed the same myelolytic action as the same urine without sterilization.

Next, the attempt was made to concentrate and to purify these myelolytic substances. They could be extracted from the evaporated urine with 95% alcohol and they were precipitated from this solution by pouring it into about 5 times its volume of acetone (Fig. D). Dioxane extracted them nearly quantitatively from these acetone precipitates, indicated by the fact that the extracted urines, if incubated with rat spinal cord, did not fragment myelin sheaths. Based on these observations the following method is used at present: The residue of one liter of evaporated urine is dissolved in 50 cc of water and shaken with 250 cc of dioxane. After filtration through a Buechner funnel, the dioxane is separated in a separation funnel; the water solution again is shaken with 100 cc of dioxane and both dioxane solutions are combined. They are evaporated *in vacuo* to a thick syrup, redissolved in 5 cc absolute alcohol and slowly poured into 100 cc of water-free ether. The resulting precipitate is separated by centrifugation. In contains about one-half of the myelolytic substances and can be further purified by redissolving it in alcohol and precipitating it again by ether. A similar method has been used by Jones and McNulty for the separation of bile salts.[7] The relative amount of myelolytic substances in the supernatant fluid was determined by incubating varying amounts (200, 300, and 500 mg) of its residue, following evaporation, with spinal cord and by comparing the myelolytic effect with that produced by the same amounts of precipitate. The myelolytic activity was greatly reduced during these different processes, in the same way as exposure to air rapidly diminished the activity of acetone precipitates.

The yellowish precipitate obtained from the dioxane extracts was very hygroscopic. It dissolved readily in alcohol and dioxane; 500 mg of it, dissolved in 5 cc of saline solution and incubated with approximately 30 mg of rat spinal cord gave a positive myelolytic reaction. The substance was strongly surface active, and, compared with sodium taurocholate of equal concentrations, it possessed about one-fifth of the surface reducing activity of this substance; *e. g.*, 58 mg dissolved in 6 cc of water had a surface tension of 65.5%, as measured with the stalagmometric method used by Allen for the measurement of surface tensions of urines.[8] The color reactions for cholic acid and desoxycholic acid and for urobiline were negative.

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[7] Jones, K. K., and McNulty, M. C., personal communication.

[8] Allen, G. D., *J. Biol. Chem.*, 1915, **22**, 505.

The attempt will be continued further to purify and to analyze this substance.

Summary. A substance which acted destructively upon the myelin sheaths of rat spinal cord could be demonstrated in the urines of patients suffering from disseminated sclerosis. It was more frequently observed during periods of exacerbations than during periods of remissions. Similar myelolytic substances were also observed in cases of post-encephalitic Parkinsonism, but they were absent in syphilis and in other diseases of the central nervous system. This myelolytic substance could be isolated from the concentrated urines by extraction with dioxane.

13283

Relation of Blood Lactic Acid and Acetone Bodies to Uric Acid in Pre-Eclampsia and Eclampsia.*

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Pre-eclampsia is a disease of the last trimester of pregnancy characterized by hypertension, albuminuria and edema. It may develop into eclampsia, which is distinguished from the pre-eclampsia by the occurrence of convulsions. Hyperuricemia is the most consistent blood chemical finding in these diseases.[1, 2] It has been attributed to a disturbance of uric acid metabolism in the liver. Quick[3] has suggested that it may result from an elevated blood lactic acid known to occur in eclampsia,[4-7] because lactic acid ingestion causes uric acid retention. Evidence against this theory is that the blood lactic acid in eclampsia rises mainly after the convulsions,[5, 6] whereas the blood uric acid is continuously elevated.

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* Supported by a grant from the John and Mary R. Markle Foundation.

[1] Cadden, J. F., and Stander, H. J., *Am. J. Obst. and Gynec.*, 1939, **37**, 37.

[2] Schaffer, N. K., and Stander, H. J., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 180.

[3] Quick, A. J., *J. Biol. Chem.*, 1935, **110**, 107.

[4] Stander, H. J., Radelet, A. H., *Bull. Johns Hopkins Hosp.*, 1926, **38**, 423.

[5] Zweifel, E., and Scheller, R., *Zentralbl. Gynäkol.*, 1927, **51**, 655.

[6] Bokelmann, O., *Arch. Gynäkol.*, 1927, **129**, 802.

[7] Stander, H. J., and Cadden, J. F., *Bull. Johns Hopkins Hosp.*, 1930, **47**, 382.

The level of the blood lactic acid in pre-eclampsia would be critical evidence for or against this theory because there are no convulsions to affect the lactic acid. Previous evidence on the blood lactic acid in pre-eclampsia[6, 7, 8] has been conflicting; therefore we have re-investigated this problem. Blood acetone bodies were also determined, because Quick[9] has shown that acetoacetic acid likewise causes uric acid retention.

Methods. Blood was collected in bottles containing 2 mg each of potassium oxalate and sodium fluoride per cc of blood, the latter added to inhibit lactic acid formation from glucose. Whole blood tungstic acid filtrates were prepared by adding 1 cc of 10% sodium tungstate per cc of blood and slowly adding 8 cc of N/12 H_2SO_4. Lactic acid was determined by the colorimetric method of Barker and Summerson.[10] Total acetone bodies were determined[†] by distillation from the acidified blood filtrate after bichromate oxidation and determination of the distilled acetone by the Behre method.[11] Uric acid was determined by the Folin method.[12]

Results. Ten normal pregnancies in the last trimester, 7 cases of pre-eclampsia and 2 cases of eclampsia were studied (Tables I and II). The average blood lactic acid in antepartum normal pregnancy was 11.0 mg % (9.1-12.3) and in antepartum pre-eclampsia was 12.9 mg % (8.0-18.6). The average blood acetone bodies in normal pregnancy was 1.3 mg % ($<$0.1-2.7) and in pre-eclampsia were 0.8 mg % ($<$0.1-2.6). The average blood uric acid in pre-eclampsia was 4.5 mg % and in normal pregnancy was 3.0 mg %. No significant change in either blood lactic acid or acetone bodies was associated with the elevated blood uric acid in pre-eclampsia.

The two cases of eclampsia were included to indicate the effect of convulsions on the blood constituents. In the intrapartum case in the first stage of labor and 25 minutes after a convulsion the blood lactic acid was 57.1 mg % as compared with 9.6 mg % for a corresponding intrapartum normal pregnancy. Winkler and Hebeler[13] have reported that the average blood lactic acid is about doubled in early normal labor, therefore, a part of this increase was probably associated with labor. We found no such increase in lactic acid in the intrapartum normal pregnancy, but obviously no conclusion can be drawn from a single case. In the postpartum case 2⅓ hours

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[8] Stander, H. J., and Radelet, A. H., *Bull. Johns Hopkins Hosp.*, 1926, **39**, 91.

[9] Quick, A. J., *J. Biol. Chem.*, 1932, **98**, 157.

[10] Barker, S. B., and Summerson, W. H., *J. Biol. Chem.*, 1941, **138**, 535.

[†] To be published elsewhere by W. H. S.

[11] Behre, J. A., *J. Biol. Chem.*, 1940, **136**, 25.

[12] Folin, O., *J. Biol. Chem.*, 1933, **101**, 111; 1934, **106**, 311.

TABLE I.
Blood Constituents in Normal Pregnancy. Values in mg%.

	Lactic acid	Total acetone bodies (as acetone)	Uric acid
1. Antepartum	12.3	1.7	2.4
2. ”	11.1	1.5	2.4
3. ”	11.5	1.9	1.9
4. ”	10.6	<0.1	2.2
5. ”	10.8	1.5	4.6
6. ”	9.1	0.9	3.6
7. ”	12.1	2.7	4.2
8. ”	10.2	0.5	2.6
Avg	11.0	1.3	3.0
1. Intrapartum	9.6	7.2	3.1
2. Postpartum (6 hr)	12.1	0.4	2.1
Avg	10.9	3.8	2.6

TABLE II.
Blood Constituents in Pre-eclampsia and Eclampsia. Values in mg%.

Case	Lactic acid	Total acetone bodies (as acetone)	Uric acid	Min. after convulsion
1. Mild pre-eclampsia, antepartum	10.7	1.8	5.6	
2. ” ” ”	18.6	<0.1	3.5	
3. ” ” ”	10.8	<0.1	3.9	
4. ” ” ”	8.0	2.6	4.9	
5. Severe ” ”	17.6	0.2	4.9	
6. ” ” ”	16.2	0.3	3.7	
7. ” ” ”	8.5	0.8	5.3	
Avg	12.9	0.8	4.5	
1. Eclampsia, intrapartum	57.1	1.3	5.1	25
2. ” , postpartum (7 hr)	16.0	3.1	5.1	2⅓ hr
Avg	36.6	2.2	5.1	

after a convulsion the blood lactic acid was 16.0 mg % as compared with 12.1 mg % for a normal pregnancy at about the same postpartum time of 6-7 hours, indicating that the blood lactic acid was already close to normal.

Our present data on the blood acetone bodies in eclampsia are insufficient for a conclusion. It has been previously reported[6, 7] that they are increased by the convulsions. The high value of 7.2 mg % in the intrapartum normal pregnancy indicates that they are increased by labor, a finding that has been reported before.[13]

Discussion. Inasmuch as there is no significant difference in the blood lactic acid and acetone bodies between pre-eclampsia and normal pregnancy, the hyperuricemia of pre-eclampsia can not be attributed to an elevation of these constituents. It is true that there are 3 relatively high lactic acid values in the pre-eclamptic group, but the average value is only 17% above that for the normal pregnant group. Moreover, 2 of these high lactic acid values are associated with the 2 lowest uric acid values and three of the highest uric acid values are associated with the lowest lactic acid values.

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[13] Winkler, H., and Hebeler, F., *Arch. Gynäkol.*, 1939, **168**, 64.

Since the hyperuricemia of pre-eclampsia is not due to an elevation of blood lactic acid or acetone bodies, it follows that the hyperuricemia of eclampsia also is not primarily due to this because pre-eclampsia and eclampsia are believed to be a single disease entity. However, a rise of these constituents in the blood due to the convulsions in eclampsia may possibly augment the pre-existing hyperuricemia.

Summary. The average blood lactic acid in 7 cases of pre-eclampsia was 12.9 mg % (8.0-18.6) as compared with 11.0 mg % (9.1-12.3) in 8 cases of normal pregnancy. The average blood acetone bodies in the pre-eclamptic cases was 0.8 mg % (<0.1-2.6) as compared with 1.3 mg % (<0.1-2.7) for normal pregnancy. The average blood uric acid for the pre-eclamptic group was 4.5 mg % and for normal pregnancy was 3.0 mg %. The rise in blood lactic acid in eclampsia occurs mainly after convulsions. The hyperuricemia of pre-eclampsia and eclampsia can not be primarily attributed to an elevation of blood lactic acid or acetone bodies.

13284

A New Salmonella Type, *Salmonella illinois*.*

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The Salmonella type to be described is represented by 7 cultures isolated from 3 outbreaks of animal disease. *Salmonella illinois* has been recognized in cultures isolated from hogs in Illinois by Dr. Robert Graham, from Hungarian partridges in Michigan by Miss

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* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

Virginia Stoney, and from turkeys in Minnesota by Dr. B. S. Pomeroy. No other microörganism was found associated with the bacillus in any of the animals. The biochemical and serological characteristics of the seven cultures were identical. They were motile rods which conformed to the definition of the genus Salmonella in their morphological, tinctorial and biochemical properties. All produced hydrogen sulphide, failed to produce indol and did not liquefy gelatin. Arabinose, maltose, rhamnose, xylose, dulcitol, inositol and sorbitol were fermented with the production of acid and gas. Lactose, sucrose, salicin and adonitol were not attacked. Dextro-tartrate, levo-tartrate, citrate and mucate were fermented by the bacilli but meso-tartrate was not utilized.

On serological examination it was found that the bacilli be-

TABLE I.
Somatic Antigens of *S. illinois.*

	Serums							
	S. senftenberg (I III XIX) absorbed by				*S. anatum* (III X) absorbed by			
Antigens	Unabsorbed	*S. anatum* (III X)	*S. newington* (III XV)	*S. illinois*	Unabsorbed	*S. senftenberg* (I III XIX)	*S. newington* (III XV)	*S. illinois*
S. senftenberg	2000	1000	1000	1000	500	0	0	200
S. anatum	200	0	0	100	1000	200	200	500
S. newington	200	0	0	50	500	0	0	200
S. illinois	100	0	0	0	200	0	0	0

	Serums								
	S. newington (III XV) absorbed by					*S. illinois* absorbed by			
Antigens	Unabsorbed	*S. senftenberg* (I III XIX)	*S. anatum* (III X)	*S. illinois*	*S. illinois* + *S. anatum*	Unabsorbed	*S. senftenberg* (I III XIX)	*S. anatum* (III X)	*S. newington* (III XV)
S. senftenberg	500	0	0	200	0	200	0	0	0
S. anatum	500	50	0	200	0	200	0	0	0
S. newington	2000	1000	500	1000	500	1000	200	200	0
S. illinois	1000	200	200	0	0	2000	1000	1000	1000

Figures indicate highest dilution in which agglutination occurred.
0 indicates absence of agglutination in a dilution of 1 to 25.

longed to Group E of the Kauffmann-White classification. Alcohol-treated suspensions were agglutinated by all serums containing agglutinins for antigen III. They were unaffected by serums representative of other groups of the diagnostic schema. The O antigens were examined more closely by agglutinin absorption tests, the results of which are given in Table I. It was found that *S. illinois* contained only a portion of antigen III and a portion of antigen XV. In addition it contained a strong somatic factor peculiar to itself. To the latter antigen the symbol XXXIV is assigned. The O antigens of *S. illinois* may be expressed as (III) (XV) XXXIV.

Examination of the flagellar antigens revealed that the organism was diphasic. Phase 1 of *S. illinois* was flocculated to titer by serum derived from phase 1 of *S. glostrup*. Likewise phase 1 of *S. glostrup* was flocculated to titer by serum derived from phase 1 of *S. illinois*. In reciprocal absorption tests it was found that phase 1 of each of these 2 organisms completely removed agglutinins from serum derived from phase 1 of the other. Phase 1 of *S. illinois*, like that of *S. glostrup*, is represented by the symbol z_{10}.

The non-specific antigens contained in phase 2 of the bacilli were tested with absorbed serums containing agglutinins for factors 2, 3, 5, 6, and 7 respectively. The organisms were agglutinated only by serum containing agglutinins for factor 5, which indicates that the organism lacks antigen 3 and has the non-specific formula 1, 4, 5 . . . . This assumption was confirmed by absorption tests. After absorption with *S. illinois*, serum derived from phase 2 of *S. cholerae-suis* contained a slight residue of agglutinins for phase 2 of *S. cholerae-suis* (1, 3, 4, 5 . . .), *S. thompson* (1, 3, 4, 5 . . .) and *S. newport* (1, 2, 3 . . .); but contained no agglutinins for phase 2 of *S. reading* (1, 4, 5 . . .) or *S. muenster* (1, 4, 5 . . .).

Summary. A new Salmonella type, *S. illinois*, is described. It has been found in hogs, partridges and turkeys. The antigenic formula of the bacillus is (III) (XV) XXXIV:z_{10}—1, 5 . . . .

13285

Protective Values of Various Types of Vi-Phage on Experimental Typhoid Infection in Mice.

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The importance of the kind of phage employed in determining the efficacy of phagotherapy in experimental typhoid infection has been indicated by the work of Asheshov, Wilson and Topley.[1] These workers have found that 2 strains of Vi-phages specific for V-form *B. typhosus* were effective in preventing deaths of infected mice, while a non-specific phage, capable of lysing both V- and W-forms of *B. typhosus in vitro,* was ineffective. More recently Craigie and Yen[2] found that the Vi-phages can be grouped into 4 serological types which are all specific for V-form *B. typhosus* but differ in their thermal death point, particle-size and relative lytic activity for various strains of V-form *B. typhosus.* In view of the latter findings, it is of practical interest to ascertain whether or not these serological types of Vi-phages would differ in their protective action on experimental typhoid infection. In this communication our observations are limited to the determination of their relative efficacy on mice experimentally infected with a strain of V-form *B. typhosus.*

The Ty2 strain was used throughout in this experiment. For infecting the animals, 3- to 5-hour broth cultures were prepared from an 18-hour agar growth. When sufficient growth was reached, the broth culture was chilled in ice-water and diluted with ice-cold broth to an opacity of 10% No. 4 standard of a Pulfrich photometer. At this opacity the broth culture contained 100 million organisms per milliliter. The phages employed were the original strains of Type I, II, III, and IV Vi-phages isolated by Craigie and Yen.[2] All these phages were propagated on the Ty2 strain. The titers of the phages were expressed in plaque-units, being the number of discrete plaques as determined on an agar-surface growth except for the type I Vi-phage in which instance the plaque-counting was made by the pour-plate technic,[3] using 0.7% agar so as to render the plaque more easily visible.

Normal mice weighing from 15-18 g were kept in separate groups

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[1] Asheshov, I. N., Wilson, J., and Topley, W. W. C., *Lancet*, 1937, **1**, 319.

[2] Craigie, J., and Yen, C. H., *Canad. Pub. Health J.*, 1938, **29**, 448.

[3] Yen, C. H., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1006.

TABLE I.
Survival-rate of Infected Mice with and without Phagotherapy.

	Phage treated					
		Types of Vi-phage				
Tests	Phage units given	I	II	III	IV	Controls
1	5 x 10^8	2*	4	2	—	1
2	7.5 x 10^8	2	7	7	7	0
3	1.5 x 10^9	1	8	8	8	0

*The figure represents number of animals surviving over a week from each group of 10 animals tested.

of 10 each. All the animals were given intraäbdominal infections of 100 million organisms of the Ty2 culture. In the phage-treated groups, Vi-phages of various potencies were given in 1 cc amounts subcutaneously immediately following the infection. The animals were kept under observation for a week and the number of survivals are summarized in Table I.

Results. Death of the animals usually occurred within 48 hours after infection and in the majority of instances within 24 hours. The Type I Vi-phage was found to show no significant protective action under the experimental conditions. On the other hand the Type II, III, and IV Vi-phages were found to be equally effective in preventing about 70 to 80% of the infected mice from dying, when the phage-units given approximated 8 to 15 times the number of organisms contained in the infecting dose. Ordinary broth and Vi-phages inactivated by immersing in boiling water for 10 minutes were separately given to a few groups of infected mice but all failed to show any beneficial effects.

Comments. It may be pointed out here that in contrast to the other 3 Vi-phages the Type I Vi-phage produced very minute plaques on agar-surface growths, and highly potent preparations were obtained with difficulty. Furthermore, using similar concentration of phage-units as the other types, the clearing of a given broth culture was not only slower but also less complete. It is of interest, therefore, to correlate the lack of demonstrable protective action *in vivo* with its slower rate of lysis of the organisms *in vitro*. As the phagotherapy is given subcutaneously following infection, the evaluation of the protective action of these phages are rather more strict than if they were given intravenously as employed by others.[1] Therefore the non-protective action of Type I Vi-phage is relevant only under the specified conditions. Also the phage-susceptibility of various strains of V form *B. typhosus* vary greatly not only with the type of Vi-phage used for testing but also with the strain of organisms employed for the propagation of the phages.[2, 4] Hence our findings here cannot be justifiably generalized for the *in vivo* action of these phages towards all strains of V form *B. typhosus.* These observations, however, are sufficient to demonstrate a significant difference in the relative efficacy of these phages on experimental typhoid infection in mice.

Conclusion. Of the 4 serological types of Vi-phage propagated on Ty2 strain of V form *B. typhosus,* only the Type I Vi-phage failed to show noticeable protective action under the specified experimental conditions. The Type II, III and IV were found to be equally effective in their protective action.

13286 P

Influence of Specific Soluble Substance on Course of Experimental Pneumococcus Pneumonia.

Edward Bigg. (Introduced by O. H. Robertson.)

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The specific soluble substance (SSS) of pneumococci has been shown by many workers to inhibit the action of natural or acquired antibodies, either partially or completely. The purpose of this investigation was to determine the effect of this material* on the course of experimental canine Type I pneumococcus pneumonia when administered in sufficient quantity to completely neutralize the humoral defense mechanism.

Healthy 10K dogs were used in this study. They were infected by the intrabronchial route using a uniform dose of 0.001 cc of actively growing Type I pneumococci suspended in 1 cc of starch-broth mixture.[1] This quantity was employed since in the experience of this laboratory such dose seldom produces bacteremia and characteristically results in a localized lobar lesion. Five control dogs in this series confirmed this finding. Hourly samples of blood were taken for culture and serum pneumococcidal-promoting power.

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[4] Craigie, J., and Yen, C. H., *Trans. Roy. Soc. Canada,* 1937, **37**, Sec. V, 79.

* The SSS used in this study was supplied by the Lederle Laboratories, Inc., through the courtesy of Dr. W. G. Malcolm.

[1] Terrell, E. E., Robertson, O. H., and Coggeshall, L. T., *J. Clin. Invest.,* 1933, **12**, 393.

Observations of the leukocyte count and temperature were recorded at these intervals. Chest roentgenograms were taken in all dogs 24 hours after infection and repeated as indicated.

Using normal dog serum in serum-leukocyte mixtures,[2] pneumococcidal action was completely abolished in a dilution of 1:250 SSS. To obtain this concentration in a 10K animal it was necessary to use 1.5 g of SSS. When this dose was administered intravenously the dog immediately developed shock and died within a few hours. The largest intravenous quantity that could be given, compatible with life, was 0.30 g dissolved in 50 cc Locke's solution. This dose produced an immediate decrease in leukocytes to leukopenic levels, followed by a progressive rise until marked leukocytosis was present within 8 to 24 hours. The temperature curve paralleled the white blood cell count. Concentration of the blood, tachycardia and fall in pulse pressure occurred. The effect on the pneumococcidal-promoting power of the serum was somewhat variable. Of 32 dogs tested, 18 had complete abolition of this property, 4 had moderate impairment, 7 minimal inhibition and 3 had no demonstrable deviation from normal.

In 11 dogs 0.3 g of SSS was injected 15 minutes after the initiation of infection. Of this group 7 developed overwhelming bacteremia resulting in death. Although 4 dogs recovered, only one maintained a sterile blood stream, the remaining 3 animals showed a transient bacteremia.

The next procedure was to inject 0.30 g of the polysaccharide, allow sufficient time (12 hours) for the body to overcome the shock of its administration and infect the animal while a high titer of SSS was present in the blood stream. Of 9 dogs so treated, 4 ran an uncomplicated course; 2 survived a transitory bacteremia; and 3 developed progressively increasing bacteremia resulting in death.

Five animals were infected and 0.30 g SSS given after a period of 16 hours had elapsed. Well localized lobar lesions were demonstrable in chest X-rays taken before the introduction of polysaccharide. In this series one dog developed a mild transient bacteremia; all recovered.

Lesions in the bacteremic dogs differed strikingly from the controls. The area of infection was circumscribed and limited to a small portion of the infected lobe; cellular reaction was minimal. Phagocytosis, as indicated by the number of intracellular organisms, was markedly lessened and only slight intra-alveolar edema was present. Such small lesions have never been observed previously in experimental canine pneumonia accompanied by bacteremia. In such instances consolidation of at least one lobe occurs and the usual finding is that of multilobar involvement.

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[2] Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1924, **39**, 219.

To control shock effect as a possible factor in these results, nonspecific protein shock was induced in 5 dogs, at the time of infection, by the intravenous injection of peptone and precipitable substance from uninoculated culture media. One developed a mild transient bacteremia; all recovered. Heterologous SSS (Type II and III) was given to 4 dogs. Although bacteremia occurred in all cases (one death in an animal developing multilobar involvement and empyema) the lesions produced were similar to those of the control series and involved the entire infected lobe. Only one dog had complete loss of serum pneumococcidal-promoting power which occurred at the time of a demonstrable empyema 24 hours post infection.

Conclusions. Intravenous administration of 0.1 g or more per kg of SSS produces death in normal dogs. 0.03 g per kg was found to be compatible with life. Injection of this quantity produced marked alteration in the usual course of the disease. Although serum-leukocyte mixtures did not always show complete abolition of blood pneumococcidal power, the presence of this substance in the blood and tissues resulted in overwhelming bacteremia, inability of the dog to localize infection and impaired phagocytosis in the pulmonary lesion.

13287 P

Neutralizing Antibodies Against St. Louis and Western Equine Encephalitic Viruses in Horses and Fowl.

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It had previously been noticed that certain serums from healthy domestic fowl in California[1] showed neutralizing antibodies against the Br strain of western equine encephalomyelitic virus. Serums from areas where this disease is endemic appeared to yield more positive results than those from supposedly non-endemic regions. A correlation had also been observed with human serums between the presence of antibodies for both the western equine virus and that of the St. Louis encephalitic type. About 26% of 69 serums of encephalitic patients in the Central Valley areas of California were positive to both viruses.[2]

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* Aided by a grant from the National Foundation for Infantile Paralysis.

[1] Howitt, B. F., *J. Inf. Dis.*, 1940, **67**, 177.

To determine if a similar relationship existed with animals, neutralization tests were performed on serums of domestic fowl and horses collected from endemic and presumably non-endemic areas of California.[†] The same technic and the same strains of virus (Br and St. Louis) were used as previously described,[1] the serums being considered positive or weakly positive according to the number of virus dilutions neutralized.

It was interesting to find that antibodies against the St. Louis virus were present in the blood of many horses and domestic fowl and that, with but a few exceptions, they were in association with the neutralizing substance against the western equine virus. Of 43 unvaccinated normal horses and 2 recovered animals from the endemic regions of Kern, Fresno and Yolo Counties, all (95.3%) except 2 showed neutralizing antibodies against the western equine virus. With the exception of 10, the serums were positive to the St. Louis virus as well, but none was positive to the latter alone. Serums were tested from 19 horses vaccinated against the equine strain in a Northern California area. All were positive to the homologous virus but were negative to that of the St. Louis. Of 3 unvaccinated animals 2 were negative to both strains and one weakly positive to the equine. Many of these horses had been imported from the East, and therefore had not been long exposed to the western environmental factors.

Of 12 horses from coastal areas where encephalomyelitis was rarely reported, 4 (33.3%) showed antibodies for the equine virus and 2 for the St. Louis (16.6%). It was among the latter group that one serum was positive to this virus alone. Nine unvaccinated horses were bled from the supposedly non-endemic desert portion of Kern County. Four were negative to both viruses and 5 positive to the equine strain, while all were negative to the St. Louis.

By far the greatest proportion of serums positive for both viruses was obtained from known endemic regions, 100% of the 21 Kern County horses in the farming areas being positive to the equine and

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[2] Howitt, B. F., *Am. J. Pub. Health*, 1939, **29**, 1083.

[†] The authors are indebted to Dr. N. Twisselmann of the Kern County Health Department and to Drs. W. R. Hinshaw and H. Cameron of the U. C. Agricultural Station at Davis, California, for the collection and contribution of much of the material used in this study.

90.9% to the St. Louis virus as well, while at Davis in Yolo County 100% of the 15 unvaccinated horses had antibodies for the equine and 78.5% for both viruses.

The same relationship in antibody formation was found for the serums of domestic fowl as for the horses. Of 96 chicken serums from known endemic regions, 43 (46.8%) were positive against the equine virus and 23 (25.5%) of 90 serums tested were positive against the St. Louis strain. All of the birds were over 1 or 2 years of age and had lived through at least one summer, when an inapparent infection might have occurred. Of 65 normal chickens, mainly White Leghorns, from non-endemic coastal areas, including 28 hatched at the laboratory, only 5 (7.0%) showed antibodies for the equine virus and none for the St. Louis. All the positive birds came from one place. None of those from the 3 other regions nor those hatched in the laboratory was positive. Ten old hens survived an intracerebral injection of the St. Louis strain but one 3-weeks-old chick and one English sparrow both succumbed. The virus was recovered from the brains of each bird.

The largest number of serums positive to the equine virus came from chickens of varied breeds on small farms in Kern County, where either human or equine cases of encephalomyelitis had occurred. Only those birds were bled that had been present at the time of the illness. Twenty-two (66.6%) serums out of 33 protected against the equine virus, while 12 (42.8%) gave protection against the St. Louis strain. All those positive to the latter were also positive to the equine virus. Blood from 12 chickens of non-encephalitic homes in the city of Bakersfield, Kern County, showed 4 (33.3%) positive tests for the equine virus and one weakly positive for the St. Louis strain as well.

The 27 serums from the endemic Davis area only showed 4 (14.8%) positive to the equine strain alone and 3 (11.1%) to the St. Louis. These birds had largely been kept within breeding pens, not roaming at large. On the other hand 13 turkeys from Davis that were allowed more freedom to range outside, showed 8 positive serums (61.5%) for the equine virus and none for the St. Louis. The serums of one out of 5 ducks, 2 out of 5 pheasants, and of 2 peacocks from the endemic areas were positive for the equine virus. The latter also had antibodies for the St. Louis strain and one duck was weakly positive for this virus alone.

It is difficult to decide as to the significance of the antibodies to both of these viruses among apparently healthy domestic animals, especially when it had been found[1] that fowl without neutralizing substances were often insusceptible to either virus. Further work is being done in this regard, however, and a detailed discussion will be presented later. At present, there is apparently a correlation between certain environments contributing the clinical disease in man and horses and the development of antibodies to both viruses in the blood of fowl and horses. A common vector or common reservoir may be responsible for both apparent and inapparent infections.

Because of the presence of neutralizing substances for the St. Louis virus among relatively insusceptible animals, one may surmise that man is not the only host for this disease, which may be more widespread in nature than supposed. Harrison and Moore[3] have found young chicks somewhat susceptible, Pearson[4] has reported that although clinical symptoms are absent, the virus may persist in the brain of chicks up to 20 days after inoculation, and Broun, *et al.*,[5] have shown that the hamster can be readily infected. These findings may alter the previous supposition that the St. Louis virus had been imported to California from the midwest states[2] and lead one to consider its possible endemicity in an environment common to the virus of equine encephalomyelitis. Recent observations have shown that similar conditions may occur in the Yakima Valley, Washington,[6] and since this work was done, Philip, Cox and Fountain[7] and Cox, Philip and Kilpatrick[8] have not only found neutralizing antibodies to the St. Louis virus in horses but have proven the latter experimentally susceptible to this virus. More recently Hammon, *et al.*,[9] have recovered the viruses of both western equine and St. Louis encephalitis from the mosquito, *Culex tarsalis,* taken in the Yakima Valley.

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[3] Harrison, R. W., and Moore, E., *Am. J. Path.*, 1937, **13**, 361.

[4] Pearson, H. E., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 413.

[5] Broun, G. O., Muether, R. O., Mezera, R. A., and LeGier, M., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 601.

[6] Hammon, W. McD., and Howitt, B. F., in press; Hammon, W. McD., *J. A. M. A.*, 1941, **117**, 161.

[7] Philip, C. B., Cox, H. R., and Fountain, J. H., *Pub. Health Rep.*, 1941, **56**, 1388.

[8] Cox, H. R., Philip, C. B., and Kilpatrick, J. W., *Pub. Health Rep.*, 1941, **56**, 1391.

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13288

Comparative Effects of Neutrons and X-rays on Three Tumors Irradiated *in vitro*.*

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Introduction. Per unit of ionization, neutrons have been demonstrated to be more effective than X-rays in producing biological changes. This increased effectiveness is undoubtedly due to the great difference in distribution of the ionization produced by the rays, *i. e.,* the ionization caused by neutrons is much more intense and localized than that caused by X-rays. Table I summarizes the results obtained in previous work. It will be noted that the X-ray/neutron dosage ratios, *i. e.,* ratios of the X-ray/neutron doses required to produce 50% lethal effect, vary considerably for different organisms, as well as for different tissues of the same organism, and that the ratio is always in favor of neutrons. This suggested the possibility that the X-ray/neutron ratios of the tumors would vary and thus that neutrons may be selective in their action on different tumors. The work reported here is the study of the comparative effects of neutrons and X-rays on 3 tumors of the Strong-A strain mouse,[1] a mammary carcinoma,[2] a lymphoma,[3] and a lymphosarcoma.[†] All of these tumors are very suitable for experimental work because they are easily transplantable and grow 100% in the Strong-A strain mouse.

Methods. 1. *Preparation of Tumor Particles.* A mouse with subcutaneous, bilateral tumors was killed, the tumors removed aseptically and divided into small particles weighing approximately 10 mg. These particles were first wrapped in sterile filter paper moistened with sterile, buffered, physiological saline and then wrapped in cellophane. Twenty particles were placed in each package; for each experimental point, 2 packages were irradiated (40 implants).

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* This investigation has been aided by a grant from the Dazian Foundation for Medical Research. We wish also to thank the General Electric Company for the loan of a G. E. Maximar X-ray machine used in the experiments. Acknowledgment is made of the assistance of Works Progress Administration Project No. 50-12032-c-4.

[1] Strong, L. C., *J. Hered.*, 1936, **27**, 21.

[2] Gardner, W. U., Smith, G. M., Allen, E., Strong, L. C., *Arch. Path.*, 1936, **21**, 265.

[3] Lawrence, J. H., Gardner, W. U., *Am. J. Cancer*, 1938, **33**, 112.

[†] Induced by Dr. W. U. Gardner after prolonged folliculin injections.

TABLE I.
Summary of Previous Work on Comparative Effect of X-rays and Neutrons on Various Biological Objects.

Biological effect	X-ray neutron ratio
Inhibition of growth of wheat seedlings (6)	5
’’ ’’ development of Drosophila eggs (6)	2.1
’’ ’’ ’’ ’’ Fern spores (6)	2.5
Lethal effect on mammary carcinoma (7)	5.1
’’ ’’ ’’ normal mice (8), (9)	4
’’ ’’ ’’ sarcoma 180 (8)	4
Production of lymphopenia in normal rats (10)	10
Chromosomal abnormalities (11), (12)	6
Inhibition in development of Drosophila eggs (13)	2, 2.8, 3.2
Retardation of growth of wheat seedlings (root) (13)	6.2-11.8
’’ ’’ ’’ ’’ ’’ ’’ (shoot) (13)	5.9-9
Inhibition of mitosis in rats (14,a)	6
Lethal effect on *B. mesentericus* spores (14,b)	5.3
’’ ’’ ’’ *B. coli* (14,c)	3.2
’’ ’’ ’’ bean roots (14,d)	11
Production of sterility in male mice (15)	5-6

Controls (10 implants) were prepared in the same manner and kept under as nearly the same conditions as the experimental material. After irradiation, the tumor particles were implanted subcutaneously, bilaterally into the axillary regions of mice by the trocar method (No. 13 hypodermic needles). Three hours was the limit set for the completion of the experiment, the controls being implanted last. The mice were observed weekly for 10 weeks and the number of tumor growths noted.

2. *Irradiation Technic. a. Neutron Irradiation.* Neutrons were produced by the bombardment of a beryllium target with deuterons accelerated to 16 million volts in the 60-inch cyclotron.[4] The neutron

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[4] Lawrence, Alvarez, Brobeck, Cooksey, Corson, McMillan, Salisbury, Thornton, *Phys. Rev.*, 1939, **56**, 124.

[5] Aebersold, P. C., *Phys. Rev.*, 1939, **56**, 714.

[6] Zirkle, R. E., Aebersold, P. C., Dempster, E. R., *Am. J. Cancer*, 1937, **29**, 556.

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[9] Lawrence, J. H., and Tennant, Robert, *J. Exp. Med.*, 1937, **66**, 667.

[10] Lawrence, J. H., and Lawrence, E. O., *Proc. Nat. Acad. Sci.*, 1936, **22**, 124.

[11] Marshak, A., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 176.

[12] Marshak, A., *Proc. Nat. Acad. Sci.*, 1937, **23**, 362.

[13] Zirkle, R. E., and Lampe, I., *Am. J. Roent. and Rad. Therapy*, 1938, **39**, 613.

[14] Unpublished data. (Reported in *Summary Reports from Research Centers for 1938* (London), Medical Research Council). (a-c) Spear, 1938; (d) Mottram, Gray, Read, 1939.

[15] Snell, G. D., Aebersold, P. C., *Proc. Nat. Acad. Sci.*, 1937, **23**, 374.

beam was collimated by an arrangement similar to that previously described.[5] The material to be irradiated was placed at the end of the channel through the collimator at a distance of 85 cm from the target. The packets of tumor particles were mounted on cellophane stretched over the opening of the irradiation port. A filter of 3 cm of lead was used to suppress gamma radiation. Deuteron currents of 100-150 microamperes were used. The intensity produced was about 10-15 n per minute, the n-unit being the quantity of neutrons

TABLE II.

Dose	No. of regressions	% regressions
1000 r	1	2.5
2000 r	2	5.0
320 n	10	25.0
300 n	1	2.5

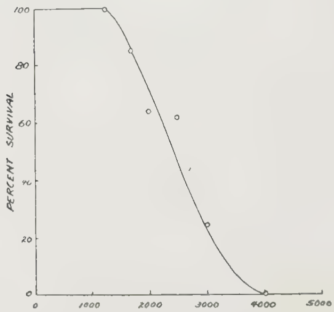
None of the regressed tumors were counted as "takes."

TABLE III.
Survival of Tumors After Irradiation.

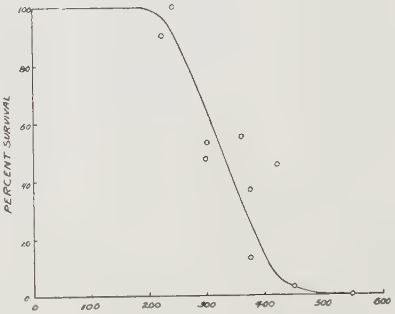
Neutrons				X-rays			
Dose in "n"	No. implants	No. takes	% takes	Dose in "r"	No. implants	No. takes	% takes
			Lymphosarcoma.				
225	40	36	90	1270	40	40	100
245	36	36	100	1700	40	34	85
300	30	16	53	2000	28	18	64
300	40	19	47	2500	40	25	62
360	38	21	55	2700	38	17	44
375	40	15	37	3000	40	10	25
375	40	5	12	4000	40	0	0
420	37	17	45				
450	40	1	2				
550	30	0	0				
			Lymphoma.				
150	40	39	97	1000	40	35	88
220	40	32	60	1200	40	32	80
300	40	18	45	1500	40	25	62
300	40	23	57	1600	40	32	80
340	40	15	37	1750	40	10	25
400	60	10	16	1800	40	6	15
475	40	0	0	2000	40	26	65
				2000	40	4	10
				2500	40	1	2
			Mammary Carcinoma.				
450	40	24	60	2500	40	39	97
495	40	34	85	3050	34	23	68
500	42	15	34	3500	40	3	7
550	40	11	27	3850	40	1	2
550	40	13	32	3400*	100	36	36
600	32	6	18	3600*	78	19	24
600	40	4	10	3600*	74	10	13
675	40	0	0	3600*	40	9	22
				4000*	38	12	31

*Earlier unpublished data.

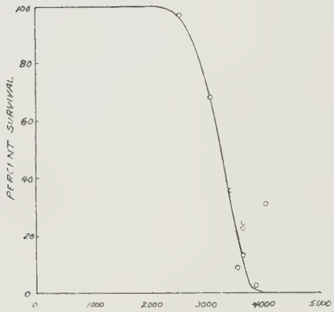
which gives the same reading on a Victoreen r-meter as does one roentgen of X-ray. The wet filter paper and cellophane wrapping around the tumor was sufficient to produce an equilibrium of the secondary ionizing particles. To avoid scattering from immediately surrounding material the tumor packages were suspended on the cellophane sheets.



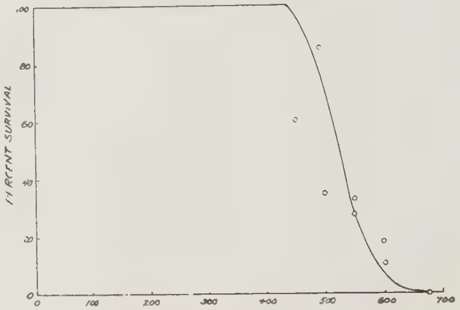
Ia DOSE IN r
IRRADIATION OF LYMPHOSARCOMA
WITH X-RAYS



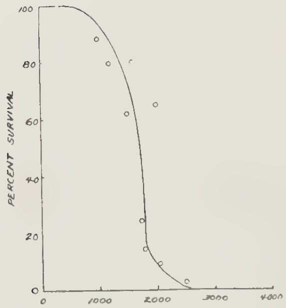
Ib DOSE IN N
IRRADIATION OF LYMPHOSARCOMA
WITH NEUTRONS



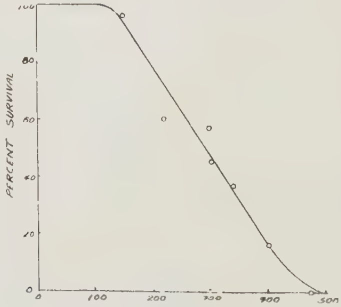
IIa DOSE IN r
IRRADIATION OF MAMMARY CARCINOMA
WITH X-RAYS



IIb DOSE IN N
IRRADIATION OF MAMMARY CARCINOMA
WITH NEUTRONS



IIIa DOSE IN r
IRRADIATION OF LYMPHOMA
WITH X-RAYS



IIIb DOSE IN N
IRRADIATION OF LYMPHOMA
WITH NEUTRONS

b. X-ray Irradiation. The source of X-rays was a General Electric 220 K.V. Maximar X-ray tube. With a target distance of 45 cm (10 cm from the end of treatment cone to packets) the output was 125 roentgens per minute. Occasionally, 0.5 mm copper filter was added to cut down the intensity; the output was then 61 r per minute. To minimize scattering from immediately surrounding material the packets were mounted on cellophane stretched between two hoops. The Victoreen r-meter was used for all dose measurements.

Results. Under control conditions, the 3 tumors grew 100%. The rate of their growth varied but slightly—lymphosarcoma appeared 2-3 weeks after inoculation, lymphoma within 2 weeks, and mammary carcinoma, 2-4 weeks. With respect to the surviving, irradiated tumors, 95% of the lymphosarcoma tumors appeared within 4 weeks after inoculation, the remainder by 5 weeks after inoculation. Of the lymphoma tumors, 99% appeared 2-4 weeks after inoculation, and 1% appeared at the sixth week. Eighty percent of the mammary carcinoma tumors which survived appeared 3-5 weeks after inoculation, the remaining 20% over a period of 6-10 weeks. Compared to the controls, the appearance of the irradiated tumors was delayed, the mammary carcinoma being more so than the other 2 tumors.

Due to the fact that none of the tumors used in these experiments spontaneously regress makes them especially valuable for experimental work. In 4 experiments regressions in the lymphoma tumor were noted in both X-rayed and neutroned material, Table II.

The results obtained for the survival of the tumors are summarized in Table III. Figures Ia and Ib show the survival values plotted for lymphosarcoma, IIa and IIb for mammary carcinoma, and IIIa and IIIb for lymphoma. When the neutron and X-ray dosages at the 50% survival point are compared, the ratio for lymphoma is 5.8, lymphosarcoma, 7.5, and mammary carcinoma, 6.1 (Table IV).

Summary and Conclusions. 1. The comparative effect of X-rays and neutrons on the survival of 3 mouse tumors was studied. 2. Both X-ray and neutron irradiation *in vitro* resulted in delaying the appearance of the tumors—mammary carcinoma being the most af-

TABLE IV.
X-ray-Neutron Ratios at 50% Survival.

Tumor	X-ray dose	Neutron dose	Ratio (r/n)
Lymphosarcoma	2450 r	325 n	7.5
Lymphoma	1700 r	290 n	5.8
Mammary Carcinoma	3250 r	525 n	6.1

fected. 3. Some cases of regressions in the growth of lymphoma were noted after neutron and X-ray irradiation. 4. When the doses at the 50% survival point were compared, the X-ray/neutron ratios of the 3 tumors, lymphoma, mammary carcinoma, and lymphosarcoma, were found to be 5.8, 6.1, and 7.5 respectively. These ratios do not seem to indicate a significant difference in the sensitivity of the tumors to the radiations, but do show that per unit of ionization neutrons are more biologically destructive than X-rays.

13289

Bone Fractures Due to Low Calcium Diets.

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From the Fleischmann Laboratories, Standard Brands Incorporated, New York City.

Several years ago[1] we reported the observation that a diet, adequate with respect to vitamin D and phosphorus but deficient in calcium, will produce a skeletal structure which is hard but light and fragile, in young growing animals. The thin-walled bones, showing no evidence of rickets, do not bend easily, have a nearly normal bone ash, and are not enlarged at the ends. Spontaneous fractures are common and are located at the point of greatest stress which varies with different species. In calves and dogs the fractures usually occur in the femur. In rabbits we find the vertebrae are the common site of fracture. Boelter and Greenberg[2] recently reported that rats on a low calcium diet frequently have spontaneous fractures of the long bones. According to our interpretation of their observations the vertebrae of rats are also susceptible to fracture.

During our study of this problem we carried out an experiment, using 5 litters of rabbits. Four of the litters were divided at weaning (3-5 weeks of age) each into 2 groups. Half of each litter was placed on a basal diet composed of whole oats 60%, alfalfa 18%, molasses 2%, whole wheat 10%, cracked yellow corn 8.5%, $NaCl$ 0.5%, Na_2HPO_4 1%, plus irradiated yeast to supply 6.5 U.S.P. units of vitamin D per gram of ration. The other half of each litter received this basal diet plus 1% $CaCO_3$. The basal ration contained

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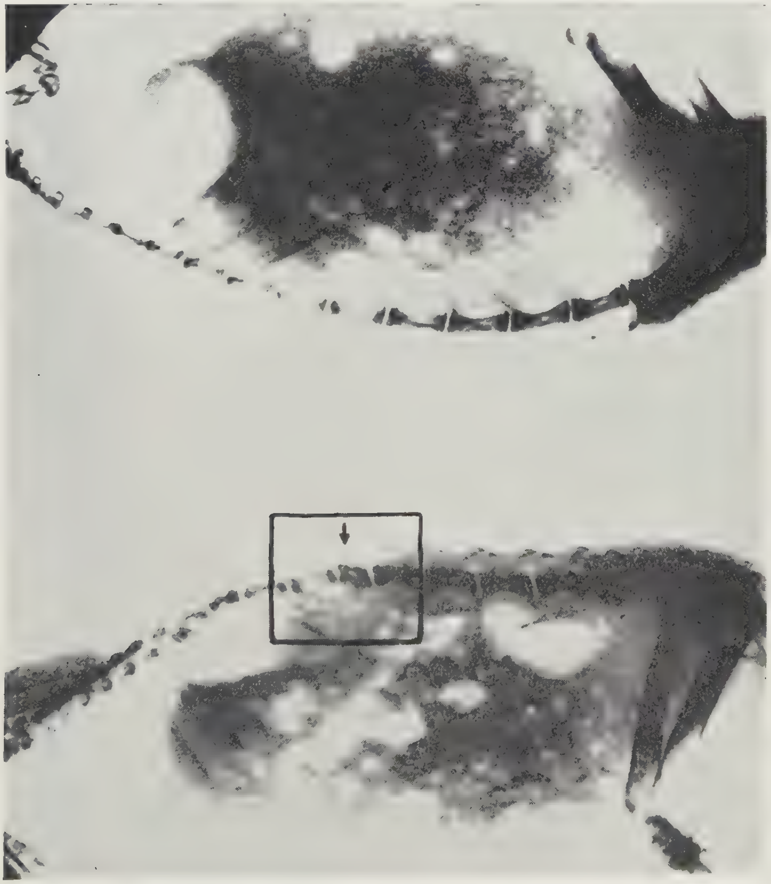
[1] Presented at the American Chemical Society meeting, Dallas, Texas, April, 1938.

[2] Boelter, M. D. D., and Greenberg, D. M., *J. Nutrition*, 1941, **21**, 61.

0.199% Ca and 0.513% P. The fifth litter was treated in the same manner, except that these animals were 8 weeks old when they were removed from their mother's pen where they had access to a commercial rabbit feed. This interval on a normal feed evidently carried the young past a critical period, since the animals of this litter placed on the basal ration remained healthy and their growth was equal to that of their litter mates fed the basal ration plus 1% $CaCO_3$.

Eight of the 9 animals from the first 4 litters on the basal low calcium diet died suddenly in 3 to 8 weeks. We X-rayed 4 of the

X-rays of Rabbits.
Comparison of Skeletal Development.
Basal diet + 1% $CaCO_3$, Group VIII.



Basal diet only, Group VII.
FIG. 1.

animals and found that one or more vertebrae had been fractured and collapsed in each instance, suggesting that vertebral fractures caused death in all cases. The crushed second lumbar vertebra of rabbit LEN, Group VII, can be seen in Fig. 1. On autopsy we found that the canal was definitely constricted on the left side. The animal showed a more complete paralysis of the left hind leg than of the right. The control litter mates fed the basal diet plus 1% $CaCO_3$ were normal throughout the experimental period, with no signs of paralysis. X-rays of several of these animals showed well developed, normal vertebral columns. (Fig. 1.)

The level of calcium in our basal diet was not drastically low (0.199%) when compared with the diets used by Boelter and Greenberg in their studies on the effect of calcium deficiency in growing rats. In their studies the rats were fed diets containing from 0.004 to 0.02% calcium. With these diets the authors describe the occurrence of paralytic symptoms which they attribute to "internal hemorrhages." The paralysis in their rats occurred suddenly, either spontaneously or following strong muscular contractions caused by galvanic shock or convulsive doses of strychnine. The paralysis always involved the hind quarters and not the forelimbs. They also noted that spontaneous fractures of the leg bones were common in the calcium-deficient animals.

It is evident from our data and that presented by Boelter and Greenberg that an X-ray study of their animals very probably would reveal that vertebral fractures resulting in compression of the spinal cord were the cause of paralysis.

Spontaneous fractures of the vertebrae in man are not uncommon. While no adequate explanation for these fractures has been given, the effects of low calcium intake may well be given consideration, especially since a diet containing as much as 0.2% calcium did not prevent poor bone development in experimental animals.

13290

Thiamin and Pyrimidine Studies on Older Subjects.*†

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Most of the studies on human vitamin requirements have been conducted on young adults or children. Very little is known regarding the vitamin requirements with advancing age. The favorable reports of the clinical application of vitamins, particularly thiamin, in geriatrics raise the question as to why such deficiencies exist. Is it reduced intake, inability to utilize, or an increased need? As a first step toward understanding this problem, a study of daily urinary excretion of thiamin in reasonably healthy older subjects was undertaken.

In this study, 4 men and 4 women were used as experimental subjects. In order to determine the state of health of the individuals, a physician‡ examined each one before and after the experimental period. All members of the group were ambulatory and in the seventh or eighth decade of life.

It was impossible to control the type or amount of food eaten by the subjects, but complete dietary records were kept during the period of the experiment. The thiamin content of the daily diets was calculated, using the tables of Munsell[1] and Booher.[2]

The experimental period of approximately 3 weeks was divided into 3 parts: A preliminary period on the usual diet, a second period during which the diet was supplemented with 2 mg of thiamin daily, and a final period during which the supplement was 4 mg of thiamin daily. The thiamin was given orally in the form of one milligram tablets at mealtime. Complete 24-hour urine samples were collected daily and analyzed for their thiamin and pyrimidine content by the fermentation method of Schultz, Atkin, and Frey.[3]

Experimental Results and Discussion. The data concerning the experiment are summarized in Tables I and II.

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* Published as Contribution No. 415 of the Massachusetts Agricultural Experiment Station.

† Aided by a grant from Standard Brands, Inc., New York City.

‡ We wish to acknowledge the coöperation of Dr. E. M. Holden, Amherst, Mass.

[1] Munsell, H. E., *Milbank Memorial Fund Quart.*, 1940, **18**, 311.

[2] Booher, L. E., and Hartzler, E. R., *U. S. D. A. Bull.*, No. 707, 1939.

[3] Schultz, A. S., Atkin, L., and Frey, C. N., *J. Biol. Chem.*, 1940, **136**, 713.

TABLE I.
Thiamin and Pyrimidine Studies.
Average Daily Values for Thiamin Intake and Thiamin and Pyrimidine Excretion.

♀	Wt	Days of	Thiamin intake		Thiamin excretion		Pyrimidine*	Thiamin retention†	
Age	Ht	exper.	Suppl. Gamma	Diet Gamma	Gamma	% of intake	excretion Gamma	Gamma	% of intake
A.P. 75 yr	130 lb 5′ 4″	1-6	0	802	82	10.2	214	505	62.9
		7-12	2000	859	246	8.6	343	2270	78.6
		13-16	2000	658	542	20.3	542	1810	68.1
		17-23	4000	829	509	10.5	463	3857	79.8
L.M. 75 yr	150 lb 5′ 1″	1-7	0	749	76	11.3	137	536	71.5
		8,9	2000	842	138	4.8	232	2472	86.9
		10-13	2000	738	386	14.1	246	2106	76.9
		14-16	4000	1111	655	12.8	569	3887	76.0
A.W. 68 yr	165 lb 5′ 7″	1-8	0	620	81	13.1	220	319	51.5
		9-13	2000	424	370	15.2	449	1605	66.2
		15,16	2000	630	445	16.9	561	1624	61.7
		17-26	4000	573	1110	24.2	757	3708	81.1
M.M. 66 yr	121 lb 5′ 3″	1-6	0	798	96	12.0	209	493	61.8
		8-11	2000	835	234	8.2	305	2296	80.9
		12-15	2000	695	465	17.2	296	1934	71.8
		16-21	4000	794	603	12.5	397	3794	79.1

*Expressed in thiamin equivalents.
†Thiamin retention = thiamin intake — thiamin and pyrimidine excretion.

TABLE II.
Thiamin and Pyrimidine Studies.
Average Daily Values for Thiamin Intake and Thiamin and Pyrimidine Excretion.

♂	Wt	Days of	Thiamin intake		Thiamin excretion		Pyrimidine*	Thiamin retention†	
Age	Ht	exper.	Suppl. Gamma	Diet Gamma	Gamma	% of intake	excretion Gamma	Gamma	% of intake
F.K. 70 yr	162 lb 5′ 10″	1-8	0	986	169	17.2	219	598	60.6
		10-15	2000	1003	246	8.1	356	2401	80.0
		16-19	2000	995	478	13.3	749	1768	59.0
		22-25	4000	1069	1120	22.0	551	3398	67.0
F.S. 74 yr	148 lb 5′ 6″	1-7	0	1020	261	25.5	219	540	52.9
		8,9	2000	842	138	4.8	232	2472	86.9
		10-13	2000	738	386	14.1	246	2106	76.9
		14-16	4000	1111	655	12.8	569	3887	76.0
F.M. 75 yr	152 lb 5′ 10″	1-7	0	1224	136	11.1	324	764	62.3
		8-10	2000	892	337	11.6	321	2234	77.3
		11-13	2000	892	366	12.6	488	2038	71.3
		14-16	4000	1268	740	14.0	650	3878	73.5
F.W. 70 yr	155 lb 5′ 8″	1-8	0	839	99	11.8	229	511	61.0
		9-13	2000	681	377	14.7	386	1918	71.5
		15,16	2000	636	629	23.9	437	1570	59.2
		17-25	4000	841	1409	29.1	710	2722	56.4

*Expressed in thiamin equivalents.
†Thiamin retention = thiamin intake — thiamin and pyrimidine excretion.

It may be seen that the thiamin content of the average daily diet for this group ranges from 620 μg to 1224 μg. This range corresponds very well with the values given by Melnick, Field, and Robinson[4] for the dietary intake of normal subjects from 25 to 35 years of age.

On the diet alone, the average daily excretion of thiamin by the women ranged from 76 μg to 96 μg; for the men, from 99 μg to 261 μg. There was considerable variation from day to day in the amount of thiamin excreted by the same individual and between individuals on essentially the same level of thiamin intake. It seems improbable that a single measure of the thiamin excretion of an individual on any one day would be a reliable index of his usual excretion. The average dietary thiamin intake for the men was higher than that for the women, which accounts in part for the larger amounts excreted. For 2 of the men, the percentage of ingested thiamin excreted was significantly higher than that for the women. This observation confirms the experience of other workers.

In all cases, when a 2 mg supplement of thiamin was given, there was a marked increase in the amount of thiamin excreted. However, the response of the individual subjects was not uniform. Six out of 8 subjects excreted about one-half to one-third as much thiamin during the first few days of the "2 mg" period as they did during the latter part of this period. This reaction might indicate that there was a definite need of the body for more thiamin than it had been receiving; the larger amount being excreted after an equilibrium had been established. The rise in the latter part of the "2 mg" period manifested itself sharply instead of being a gradual increase. The time when the rise occurred varied with the individual, ranging from the third to the seventh day. Two cases, A. W. and F. M., showed only slightly less excretion during the earlier than during the latter part of the "2 mg" period. A break was made arbitrarily in this period for these 2 people for comparison with the other subjects.

It was expected that thiamin excretion would increase when the daily diet was supplemented with 4 mg of thiamin. Four subjects, F. M., A. P., A. W., and M. M., excreted the same or smaller amounts of thiamin on the first day of the "4 mg" period than they did on the preceding day (last day of the "2 mg" period). This may indicate that there is some lag in the excretion of the ingested thiamin, although it did not manifest itself in the other 4 cases in which excretion increased immediately. The percentage of ingested thiamin excreted was not much greater than on the previous level of intake and, in some cases, it was less.

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[4] Melnick, D., Field, H., Jr., and Robinson, W. D., *J. Nutr.*, 1939, **18**, 593.

The presence of 2-methyl-5-ethoxymethyl-6-aminopyrimidine, a fraction of the thiamin molecule, in the urine is of interest and may be of more significance than we know at present. The pyrimidine may be present in the food as such and results indicate that it is also a breakdown product in the metabolism of thiamin. With increase in the thiamin intake, the pyrimidine fraction increased considerably in the urine although less than the thiamin fraction. The amount of urinary pyrimidine was remarkably constant irrespective of sex, and was usually greater than the urinary thiamin on the lower levels of intake. If the normal pyrimidine excretion can be considered above 200 μg, one subject, L. M., falls appreciably below normal. It is interesting to note that this subject showed the lowest initial response to the 2 mg supplement of thiamin. Applying the interpretation of Pollack,[5] that low pyrimidine and thiamin values indicate a protracted insufficiency of vitamin B_1, one might assume that a deficiency had existed in this case. However, this subject experienced no beneficial subjective effects from the increased thiamin intake.

If it is assumed that both the urinary thiamin and pyrimidine may be derived from dietary thiamin, the sum of these 2 fractions subtracted from intake may be considered as the amount of thiamin retained or destroyed. When this "retention" figure is calculated, it appears to increase more nearly in proportion to intake than does the excretion (see table). However, a large portion of this thiamin may be destroyed in digestion and absorption. Preliminary work indicated that, at the levels of thiamin used in this study, the loss in the feces was negligible. It would seem impossible to determine by measuring the amount excreted in the urine how much of a given amount of thiamin ingested was acutally used or stored by the body tissues.

Clinical and Subjective Observations. The physical examination of the subjects before the experiment indicated that they were all in good health and showed no evidence of any thiamin deficiency. Reëxamination after the completion of the experiment showed no changes that could be measured clinically.

The subjects were asked to note any change in their general well-being during the experimental period. Two of the 8 subjects noticed no difference in how they felt. Of the remaining 6, 2 noted definite improvement in chronic constipation, 4 felt less fatigued or were "peppier", 2 enjoyed improved appetites, and one noted an increased thirst.

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[5] Pollack, H., Ellenberg, M., and Dolger, H., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 414.

Summary. 1. The thiamin and pyrimidine excretion in the urine of older subjects, after different levels of thiamin intake, was measured by the fermentation method of Schultz, Atkin and Frey. 2. There appears to be a sex difference in the excretion of thiamin which is not apparent in the excretion of pyrimidine. 3. The response of people in this age group to increased thiamin intake is similar to that of younger people so far as excretion is concerned. 4. "Retention" calculated as thiamin intake minus thiamin plus pyrimidine excreted, was more nearly proportional to intake than was the amount excreted. 5. Six out of the 8 subjects reported some type of subjective reaction to the increased thiamin intake. Physical examinations before and after the experimental period revealed no significant changes.

13291 P

Response of Intraocular Prostatic Implants in Rabbit to Gonadotropic Hormone Administration.*

B. ROSENBERG, B. KRICHESKY AND J. A. BENJAMIN.† (Introduced by B. M. Allen.)

From the Department of Zoology, University of California, Los Angeles.

Recent work by Krichesky, *et al.*, has demonstrated the effect of testosterone propionate on prostatic implants in the eye of the male rabbit, and has confirmed earlier reports that maintenance of the transplanted accessory tissue is dependent on the normal functioning of the testes, since castration results in atrophy of the transplanted tissue.[1] In undertaking a similar experiment in which 2 different gonadotropic agents were administered, a consistent response was observed which, to the writers' knowledge, has heretofore been unreported.

Fifteen male rabbits from 4 to 6 months of age were completely prostatectomized and bits of the excised tissue implanted into the anterior chamber of the eyes following the technic described by Benjamin, *et al.*[2] Of the 60 transplants (4 per animal) 36 remained observable and in an apparently normal physiologic condition. A photographic method was employed in recording the day by day area of the transplants. The areas of individual transplants were calculated by means of a planimeter and the average value over a 2-week period was taken as 100% for that implant. An increase in area was then expressed as greater than 100% and a decrease as less than 100%.

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* Aided by research grants from the Del Amo Foundation and the Board of Research, University of California.

† B. Rosenberg and B. Krichesky, Ph.D., from the Department of Zoology, University of California, Los Angeles; J. A. Benjamin, M.D., Portland, Oregon.

[1] Krichesky, B., Benjamin, J. A., Belt, E., and Schwartz, M., *J. Urology*, 1941, **46**, 303.

All animals were injected subcutaneously 6 days per week for a 2-week period with either chorionic gonadotropin (APL) or hypophyseal gonadotropin (Anterior Hypophyseal Gonadotropic Factor).[‡] In 5 cases, after the animals had undergone such a series of injections with one hormone, they were allowed to rest for approximately one month and were then subjected to a second series of injections with the other gonadotropic agent. The results were in no way distorted by this procedure since the character and magnitude of the responses were not different from those animals given only one series of injections. Two animals were given a second series of injections of APL after having been injected previously with the same hormone.

A typical response curve (Fig. 1) serves as representative of the majority of cases, since no differences were observed with respect to APL or Gonadotropin in the amounts given (100 to 500 IU daily of APL, and 100 to 500 RU daily of Gonadotropin). During the injection period there was a rise in transplant area reaching a peak averaging 130% at 4 to 8 days after the beginning of treatment Following this initial rise there was a decrease in area, despite continued injections, to a point usually below the preinjection level. In 18 out of 22 series of injections a second rise occurred 6 to 18 days after the last injection. This "secondary response" approached and in some cases even exceeded the initial response (Fig. 1). The average magnitude of the increase was 27% with a range from 12% to 41% followed always by regression.

In all instances in which a second series of injections were carried out on a given animal but with a different gonadotropic hormone, the responses were not observably different from the initial responses to the first series of injections. Two animals received a second series

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[2] Benjamin, J. A., Belt, E., and Krichesky, B., *J. Urology*, 1940, **44**, 109.

[‡] APL and Anterior Hypophyseal Gonadotropic Factor prepared by Ayerst, McKenna and Harrison (U.S.), Limited.

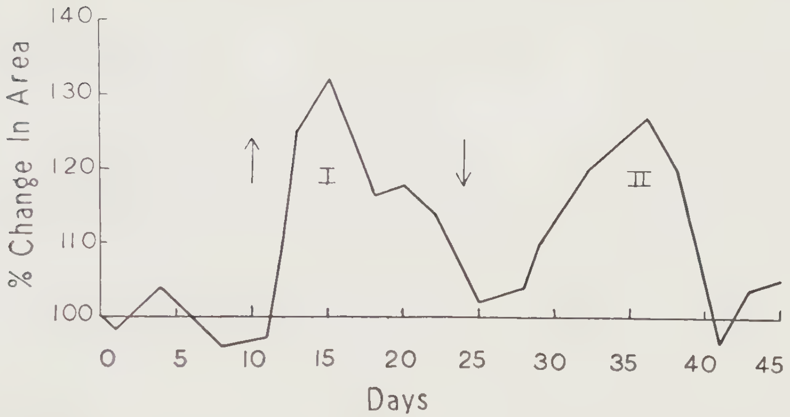

FIG. 1.

The response of intraocular prostatic implants in an untreated male rabbit to a series of 12 injections of 200 IU of A.P.L.

↑ = Injections begun. ↓ = Injections stopped.
I = Primary response. II = Secondary response.

of injections of the same hormone. No response occurred in these during the course of hormone administration but several days after the cessation of injections both animals exhibited a rise in transplant area followed by regression (Fig. 2). In these animals although no primary response was elicited by a second series of injections with the same hormone a secondary response was obtained.

Further work is in progress in an attempt to study the reaction described here as a "secondary response," a phenomenon which to the writers' knowledge has not been reported previously.

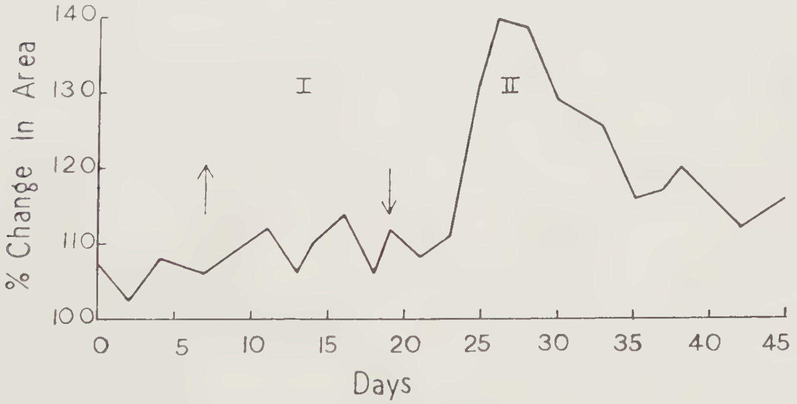

FIG. 2.

The response of intraocular prostatic implants in a male rabbit previously treated with A.P.L. to a second series of 12 injections of 200 IU of A.P.L.

↑ = Injections begun. ↓ = Injections stopped.
I = Primary response. II = Secondary response.

13292 P

Isolation of Typhus Rickettsia from Rat Mites During Epidemic in an Orphanage.

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Macxy[1] predicted that rat and mouse may be the reservoirs and tick, mite, or flea may be the transmitters of typhus fever in the southern parts of the United States. This prediction had been satisfactorily fulfilled except for the possibility of mites as vectors.[1-5] While Shelmire and Dove[6, 7] and Kodama and Kano[8] have experimentally transmitted typhus Rickettsia in laboratory animals by rat mite, no special attention was attached to this vector as an important carrier of typhus Rickettsia. In this communication, we wish to present a first instance of isolation of typhus Rickettsia from mites naturally infected and to discuss briefly its possible epidemiological significance.

A rat trapped in July, 1940, at the end of a typhus epidemic which has occurred in an orphanage was found to harbor 3 mites identified as *Liponyssus bacoti*. From them, a strain of typhus Rickettsia was established in guinea pig and white rat according to the following criteria: Fever in guinea pig and albino rat, positive Weil-Felix reaction in most of the inoculated rats, in some cases as high a titer as 1:320, recovery of Rickettsiæ on direct examination and cultivation of the *tunica vaginalis* of infected guinea pig, and finally, positive cross-immunity with a known strain of murine typhus. A second group of rat mites from the same orphanage 4 months after the epidemic showed suggestive findings on inoculation into albino rats but was not further studied. Material from the rat which harbored the first group of mites produced a suggestive typhus infection on inoculation into albino rats.

Comment. The prediction of Macxy that the mite may be a vector of typhus fever has been further supported by the isolation of a strain of typhus Rickettsia from naturally infected mites during an epidemic. However, the importance of this vector in the transmission of murine typhus cannot be easily determined. In the present epidemic, the mite-infestation among trapped rats is decidedly low, only 4 out of 24 rats caught harbored a few mites each, and no free mite was seen around the living quarters. At the same time, the rats harbored many fleas which were proved to be infected. It is, therefore, quite possible that these mites were merely secondarily infected from feeding on already infected wild rats. The available data do not permit a decision.

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[1] Macxy, K. F., *Public Health Rep.*, 1926, **41**, 2967.

[2] Dyer, R. E., Ceder, E. R., Lillie, R. D., and Badger, L. F., *Public Health Rep.*, 1931, **46**, 2481.

[3] Mooser, H., Castaneda, M. R., and Zinsser, H., *J. A. M. A.*, 1931, **97**, 231.

[4] Lèpine, P., *C. R. Acad. Soc., Paris*, 1932, **194**, 401.

[5] Sparrow, H., *Arch. Inst. Pasteur, Tunis*, 1935, **24**, 435.

[6] Shelmire, B., and Dove, W. E., *J. A. M. A.*, 1931, **96**, 579.

[7] Dove, W. E., and Shelmire, B., *J. Parasitology*, 1932, **18**, 159.

[8] Kodama, M., and Kano, M., *Kitasato Arch. Exp. Med.*, 1933, **10**, 99.

13293

Miotic Action of Lecithin and Cephalin on Denervated Iris of Cat, Monkey, Dog and Rabbit.*

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Laked erythrocytes injected intravenously, or erythrocytes hemolyzed in the blood stream produce parasympathomimetic manifestations in the cat (Bender[1]). Of these symptoms the constriction of the completely denervated iris is the most conspicuous and consistent reaction. The nature of the parasympathetic-like substance, present in the red blood corpuscles of man, monkey, rabbit, guinea pig, rat as well as in the cat is not clear. Incomplete studies made in these laboratories have suggested that the factors essential for the miosis may be associated with the phospholipids of the blood. For this reason the following comparative studies were made on the effects of certain vegetable and animal lecithins and cephalin upon the denervated iris.

Material and Methods. The animals used were cat, monkey, dog and rabbit. The iris was completely denervated by sectioning all the nerves behind the globe. Commercial preparations of lecithin and cephalin were used. Lecithin samples of vegetable, corn, soy bean, and cod fish testicle and cephalin of soy bean were supplied by the American Lecithin Company. These preparations were purified

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* This work has been aided by grants from the Josiah Macy, Jr., Foundation and the Dazian Foundation for Medical Research.

[1] Bender, M. B., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 539.

by differential extraction methods using ether extraction and reprecipitation with acetone. Artificial lecithin synthesized by method of Grün and Limpächer[2] in which stearic acid constitute each fatty acid radical was also tested.[†] Furthermore, lecithin from human erythrocytes was extracted and purified by modified differential extraction methods of Levene and Rolf,[3] MacLean and MacLean,[4] Bloor,[5] and Kirk.[6] For final testing, the phospholipid compounds were emulsified in distilled water, normal physiological saline and Locke's solutions. Volumes of one cc were injected intravenously during a period of 3 to 5 seconds. The animals were under nembutal anesthesia during the tests. In some experiments no anesthetic was used and the results were the same. The pupillary diameter was always measured with a millimeter scale.

Results. The most consistent effect obtained by the intravenous injection of the phospholipids was miosis of the cat's denervated iris. All the phospholipids mentioned above produced a conspicuous miosis in the completely denervated iris of the cat but not in that of the monkey, dog, or rabbit. The constriction occurred in every cat following the first injection and within 6 to 12 seconds after the intravenous administration of the lecithin or cephalin was begun.

Of all the lecithin studied, the synthetic preparation was the most potent; the threshold dose which produced a minimal constriction was 0.05 mg per kilo of body weight. The degree and duration of the miosis varied with the size of the dose injected; 1 mg of synthetic lecithin produced a constriction of 2 mm lasting 90 seconds, 4 mg of the same lecithin induced a constriction of 8 mm for 7 to 9 minutes. Soy bean, vegetable, corn and cod testicle lecithin and cephalin each had a miosis-inducing property, the threshold dose ranging between 0.5 mg and 1.0 mg per kilo of body weight. Intravenous injections of other colloidal emulsions and suspensions such as cholesterol (100 mg), stearic acid (10 mg), and gum acacia (60 mg) had no miotic effects.

The phospholipid miotic responses also depended on the degree of sensitiveness of the reacting organ, namely, the denervated constrictor fibers of the iris. Constriction of the pupil could be obtained in the acutely denervated iris but the longer the period following the post-ganglionic ciliary nerve section the more sensitive became the constrictor muscle to the phospholipids (Fig. 1). This sensitization diminished, however, when signs of regeneration appeared, usually the fifth week following the denervation.

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[2] Grün, A., and Limpächer, R., *Berichte Deutsch. Chem. Gesell.*, 1926, **59**, 1350.

[†] We are indebted to Dr. Harry H. Sobotka for a sample of synthetic lecithin which he obtained from Dr. Grün; and to Dr. Scharf of the American Lecithin Company for the commercial preparations of the lecithins and cephalin.

[3] Levene, P. A., and Rolf, I. P., *J. Biol. Chem.*, 1927, **72**, 587; 1927, **74**, 713.

[4] MacLean, H., and MacLean, I. S., *Lecithins and Allied Substances*, Monographs on Biochem., 1927.

[5] Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

[6] Kirk, E., *J. Biol. Chem.*, 1938, **123**, 637.

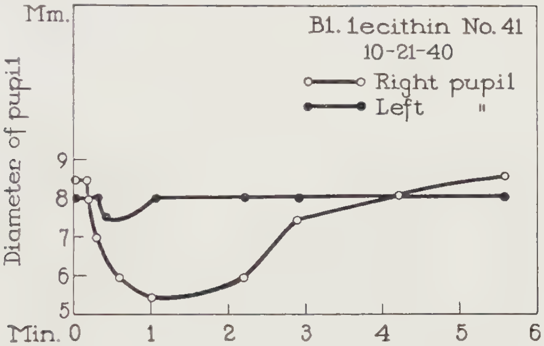

FIG. 1.

Effect of denervation on the sensitivity of the iris to phospholipids. A male cat, weighing 2.5 kilo, had its right optic and ciliary nerves sectioned on October 2, 1940. On October 19, 1940, the left optic and ciliary nerves were cut, care being taken not to injure the circulation. On October 21, 1940, under nembutal anesthesia an aqueous emulsion of vegetable lecithin, 5 mg (minimal dose for left pupil), was injected intravenously. Within 9 seconds the right pupil indicated by circles constricted rapidly while the left pupil indicated by dots constricted but slightly within 17 seconds. With a 10 mg dose the constriction became more apparent in the left pupil and more pronounced in the right. Both pupils reacted with doryl, the right constricted minimally with 1 γ, the left with 4 γ. Atropine blocked the action of doryl but not that of the phospholipid.

Atropine sulfate, injected parenterally in a dose as high as 1 mg per kilo of body weight or local application of this solution (0.1%) to the eye, had no inhibiting effect. Also, intravenous or intramuscular injections of eserine salicylate had no definite potentiating influence on the phospholipid miosis. Repeated injections of a small amount of lecithin resulted in a diminution of the miotic response with each successive dose until ultimately no miosis could be obtained (Fig. 2). Thus, when 1 mg dose is injected every 5 minutes for 6 doses the response decreases in magnitude and duration; at this point, however, the injection of 5 mg induces a miotic response which may be as great as that obtained initially.

As stated previously, none of the intravenously injected phospholipids in the concentration used with the cat and in higher concentrations had any miotic effect on the denervated iris of the monkey, dog or rabbit. Lecithin and cephalin were also without any visible effect on other denervated smooth (pupillodilator fibers) and striated muscles (facial muscles) of the cat.

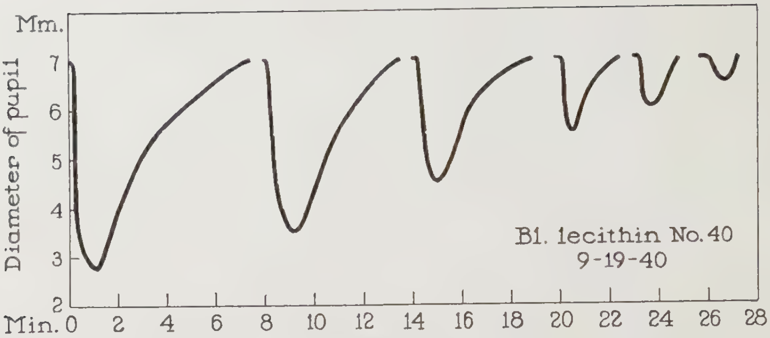

FIG. 2.

Effect of repeated intravenous injections of a constant amount of cod lecithin on the denervated iris of a cat. The denervated iris was sensitive to doryl; 1 γ produced a minimal constriction. The cat was then twice injected intravenously with 1 mg of atropine sulfate. Three dops of atropine sulfate 0.1% were also instilled in each eye. The pupil did not react with intravenous doryl as much as 100 γ per kilo after the atropinization. Cod lecithin emulsion 1 mg per kilo of body weight was then injected intravenously and the pupil rapidly constricted. Note that with each successive injection the miotic response was less in duration and degree.

Discussion. The miotic effect of the intravenously injected phospholipid emulsions is only one of a series of parasympathomimetic manifestations obtained in the cat, the other reactions not recorded in this communication being a drop in the blood pressure, slowing of the pulse rate and emptying of the bowel and bladder. These actions strongly resemble those of choline and may suggest at first glance that the reaction to phospholipid described above may be due to the hydrolyzed choline group in the lecithin. This assumption is probably incorrect for the following reasons. 1. In contrast to choline and its derivatives none of the lecithin or cephalin effect is blocked by atropine. 2. Lecithin and cephalin constrict the pupil of the completely denervated iris in the cat but not in the monkey, dog and rabbit, whereas the cholines constrict the pupil of all species. 3. Cephalin, which does not contain the choline radical, elicits a miotic reaction similar to lecithin and not influenced by atropine.

The question still remains whether the miotic effects of lecithin and cephalin are due to the phospholipids themselves or to some non-related contaminating substances. However, since synthetic lecithin is free of non-related substances which may be adsorbed by the natural phospholipids, and since this synthetic lecithin is the most potent it is reasonable to assume that lecithin itself and not some impurity is responsible for the miosis in the cat. On the other hand, a product which is used in the synthesis or possibly formed in the breakdown of lecithin such as distearoglycerophosphoric acid (phosphatidic acid) could produce similar effects. Preliminary studies with phosphatidic acid reveal that this non-nitrogen containing phospholipid has marked miotic properties in the cat.

The parasympathomimetic effects obtained with lecithin and cephalin, especially the miosis described, are almost identical with those produced by laked red blood cells of man, monkey, cat, rabbit, guinea pig and rat. As with phospholipids, the miosis elicited by hemolyzed erythrocytes can be demonstrated in the cat but not in other species (monkey, dog, rabbit), nor is the erythrocyte-miosis blocked by atropine. These data strongly suggest that the miotic property possessed by erythrocytes may actually be due to the phospholipid contents.

Summary. 1. Intravenous injections of emulsions of lecithins extracted from soy bean, corn, vegetable, cod testicle and erythrocytes of man and cephalin of soy bean produce a marked miosis in the completely denervated iris of the cat but not in that of the monkey, dog or rabbit. 2. Synthetic lecithin in much smaller concentrations yields identical results. 3. None of the phospholipid miotic effects is blocked by atropine or potentiated by eserine.

13294

Comparative Antitumoral Action of Desoxycorticosterone Acetate and Testosterone Propionate.*

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Uterine and other abdominal subserous fibroids which can be elicited by prolonged subcutaneous injections of estrogens fail to appear if testosterone propionate[1, 2] or progesterone[2, 3] is injected simultaneously with the tumorigenic estrogen. This preventive

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* This work has been aided by grants from the Jane Coffin Childs Memorial Fund for Medical Research, the Rockefeller Foundation, and Chilean friends by courtesy of Mr. Adolfo Eastman of Limache (Chile). Grants administered by Prof. A. Lipschütz. All hormones were generously supplied by Dr. Carl Miescher of Ciba Pharmaceutical Co., Basel.

[1] Lipschütz, A., Vargas, L., Jr., and Ruz, O., *Lancet*, 1939, **2**, 867.

[2] Lipschütz, A., and Vargas, L., Jr., *Endocrinology*, 1941, in press.

[3] Lipschütz, A., Murillo, R., and Vargas, L., Jr., *Lancet*, 1939, **2**, 420; Murillo, R., *Public Med. Exp.* (Chile), 1940.

antitumoral action of hormones has also been demonstrated in experiments with the simultaneous implantation of tablets of estradiol and progesterone or desoxycorticosterone acetate.[4] Experiments with tumorigenic tablets[5] also offer a good opportunity to study the question of the preventive antitumoral action of hormones from a *quantitative* point of view since knowledge of the absorbed quantities can easily be obtained by weighing the tablets.[6] The relative amount of each of the *antitumorigens* compared with that of the *tumorigens* necessary to prevent uterine and extragenital peritoneal fibroids can now be investigated. Further progress in this field has been achieved in our work with synthetic testosterone propionate and definite statements can now be made on its preventive antitumoral action compared with that of synthetic desoxycorticosterone acetate.

Tablets of estradiol (7.5 to 27.0 mg) were implanted under the skin of 18 castrate female guinea pigs (320 to 250 g). Simultaneously tablets of testosterone propionate (17.0 to 32.0 mg) were implanted on the opposite side of the same animals. They were sacrificed 55 and 56 days later. Five animals out of 18 showed uterine tumors of considerable size and 6 animals also had extragenital tumors. The total tumoral effect, according to our classification[7] varied between 0 and 6.5; the average total tumoral effect was 1.8. The tablets were cleaned from their fibrous enclosure and maintained for 20 hours at 60°C and weighed. The average absorption of estradiol was 2.5 mg, that of testosterone propionate 11.9 mg, which is equivalent to 10.0 mg of testosterone. The results are summarized in Table I and can be compared with our former findings with subcutaneous injections of testosterone propionate and with tablets of desoxycorticosterone acetate.

Table I presents evidence that conjunctive tumorigenesis is less pronounced in the present series with tablets of estradiol and testosterone propionate (series XXVI), than in experiments with tablets of estradiol alone (series XXVII). However, the preventive action of a quantity of testosterone 4 times by weight that of estradiol is not as complete as the preventive action of a quantity of desoxycorticosterone 3 times by weight that of estradiol (series XXIII). The incomplete preventive action of testosterone in the

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[4] Lipschütz, A., and Vargas, L., Jr., *Lancet*, 1941, in press.

[5] Lipschütz, A., and Vargas, L., Jr., *Lancet*, 1939, **1**, 1313.

[6] Deanesly, R., and Parkes, A. S., *Proc. Roy. Soc. B.*, 1937, **124**, 297; *Lancet*, 1938, **2**, 606; Warwick, M. H., and Parkes, A. S., *Lancet*, 1940, **1**, 406.

[7] Lipschütz, A., Vargas, L., fils, *C. R. Soc. Biol.* (Paris), 1939, **131**, 27; see also PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 164.

TABLE I.
Comparative Preventive Effect of Desoxycorticosterone Acetate and Testosterone Propionate Against Experimental Fibroids Induced with Estrogens.

No. of series	Hormones used	Duration of experiment days	Relation: Estradiol-anti-tumorigenic hormone	No. of animals			Avg. "total tumoral effect"
				Total	With uterine tumor	With uterine or extra-genital tumors, size, class 1	
	Injections:						
XIII*	Estradiol benz.	30-90	1:0	20	14	17	4.1
XIII*	Estradiol benz. and testoster. prop.	54-91	1:50	24	0	0	0.6
	Tablets:						
XXVII†	Estradiol	54-62	1:0	20	15	14	3.0
XXIII‡	Estradiol and desoxycorticost. acet.	55-63	1:3	17	0	0	0.5
XXVI	Estradiol and testoster. prop.	55-56	1:4	18	5	7	1.8

*For details see [2]. 120 to 240 γ of estradiol and 6 to 12 mg of testosterone weekly.

†For details see [4]. Tablets of 8.5 to 24.0 mg of estradiol; average absorption of estradiol—3.0 mg.

‡For details see [4]. Tablets of 8.5 to 24.0 mg of estradiol; average absorption of estradiol—2.5 mg. Tablets of 13.0 to 21.5 mg of desoxycorticosterone acet.; average absorption of desoxycorticosterone—7.7.

present series with tablets must be explained on quantitative lines; this is shown by the fact that the preventive action of testosterone is complete if greater quantities are administered by injection (series XIII). Since our experiments have been made in *castrate* females, the comparative antitumoral action of the different hormones can be considered as an exteriorization of their comparative antagonistic or antiestrogenic effect on the reacting tissues as recognized in former work with gonadal grafts.[8] This is also shown by the fact that the greater preventive antitumoral action of desoxycorticosterone compared with testosterone is coincident with 2 other striking antiestrogenic effects: *First,* the increase of the uterine weight is more effectively counteracted by desoxycorticosterone than by testosterone (average uterine wt. in estradiol series, 3.4 g; in estradiol + desoxycorticosterone series, 1.9 g; in estradiol + testosterone series, 2.6 g); and *secondly,* the vagina closed in 15 out of 17 animals of the desoxycorticosterone group whereas it remained open in all 18 animals of the testosterone group.

Summary. The preventive action of tablets of different hormones against uterine and extragenital peritoneal fibroids elicited by estradiol in the guinea pig has been compared. Desoxycorticosterone acetate is quantitatively more efficient than testosterone propionate. Desoxycorticosterone is also more efficient than testosterone in antagonizing other effects of estradiol such as opening of the vagina and increase of the uterine weight.

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[8] Lipschütz, A., and Voss, H. E., *Pflügers Arch. ges. Physiol.*, 1925, **207**, 563; Lipschütz, A., and others, *Pflügers Arch. ges. Physiol.*, 1925, **208**, 293.

13295

Effect of Vitamin D on Binucleated Cells of the Liver.*

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It has long been known that among the cells of the mammalian liver, there are some which have more than one nucleus. Most of such multinucleated cells contain two nuclei and are thus called binucleated hepatic cells. During fetal life only small numbers of binucleated cells are found, but after birth the number gradually increases until adolescence, after which time, under normal conditions, the number of cells remains constant. Each animal species has a definite percentage of binucleated cells which is equal in the various lobes of the liver and is more or less characteristic of the species.[1] Several investigators have shown that there are more binucleated cells at the periphery of the lobules than at the center.[2, 3] Although there is no conclusive evidence to support the idea, these cells are supposed to result either from hyperactivity and incomplete karyokinesis or from fusion accompanying degeneration.[4]

Szittay[3] reported that the daily administration of 3 cc of Vigantol over a period of 8 days to young rabbits led to an increase in the number of binucleated cells in the periphery of the lobule, although no changes were found in the central zone. The number of cells with 3 and 4 nuclei was slightly increased in the same region, but there was no increase in the number of mitoses. Szittay thought that his findings indicated increased activity in the peripheral zone of the lobule rather than a toxic action of vitamin D upon the liver. These observations are of interest because clinical and experimental studies suggest that the liver plays an important rôle in the action of vitamin D.[5, 6, 7] Since Szittay's experiments seemed to constitute anatomical evidence of the action of vitamin D on the liver it was decided to repeat them.

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* Presented before the American Association of Pathologists and Bacteriologists, Pittsburgh, Pa., May 22, 1940.

[1] Münzer, F. T., *Arch. f. mikr.-anat. u. Entwcklngsmechn.*, 1923, **98**, 249.

[2] Noel, R., *Compt. rend. Soc. de biol.*, 1923, **88**, 212.

[3] Szittay, Z., *Compt. rend. Soc. de biol.*, 1937, **124**, 296.

[4] Mann, F. C., in Cowdry, E. V., *Special Cytology*, Paul B. Hoeber, Inc., New York, 1932, **1**, 341.

In the first series of experiments the work of Szittay was repeated using young rats, dogs and rabbits. The rats were maintained on the Sherman diet B as modified by Smith and Bing,[8] which was supplemented with yeast and wheat germ oil. The dogs were fed Purina dog chow. The rabbits were given cabbage and carrots, which was the diet used by Szittay for his animals. All animals were allowed as much water as they would drink.

The animals of each species were divided into several groups. In the first group the rats received 1 cc and the rabbits and puppies 3 cc of viosterol. In the second group the rats received 1 cc and the rabbits 3 cc of Wesson oil. The third group consisted only of rabbits and puppies which received 3 cc of sesame oil. These substances were administered daily to the rats by pipette and to the rabbits and puppies by stomach tube. The fourth group was given thyroxin by intramuscular injection. The fifth group was used as a control and received nothing but the diet. Samples of blood were drawn before each experiment as well as at the conclusion and were analyzed for calcium and phosphorus. The animals were killed either by decapitation or intravenous injection of air, and thin slices of all organs were removed immediately, fixed in neutral 10% formalin and Zenker's fluid, embedded in paraffin, sections cut at 6 micra and stained with hematoxylin and eosin. At least 200 cells were counted in the central zone and another 200 cells in the peripheral zone of the liver lobule in each of the animals. The results were expressed as the mean number of binucleated cells per 100 liver cells.

Sesame and Wesson oils were used as controls to determine the effect of simple oils upon the liver. Thyroxin was used because it was thought that the increase in the number of binucleated cells might be due to the increase in metabolism which is known to occur with large doses of vitamin D.

Results. In young rats and dogs there was no increase in binu

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[5] Gerstenberger, H. J., *Monatschr. f. Kinderh.*, 1933, **56**, 217.

[6] Gerstenberger, H. J., cited in Griffith, J. P. C., and Mitchell, A. G., *Textbook of Pediatrics,* Philadelphia, W. B. Saunders Co., ed. 3, p. 549, 1941.

[7] Heymann, W., *Am. J. Dis. Child.*, 1938, **55**, 913.

[8] Smith, A. H., and Bing, F. J., *J. Nutrition*, 1928, **1**, 179.

cleated cells in either the central or peripheral zones of the lobule following the administration of vitamin D or of the control substances.

In rabbits, however, changes similar to those described by Szittay were observed (Table I). After the fourth day of administration of viosterol the number of binucleated cells in the peripheral zones of the lobules was definitely increased. It should be emphasized that the increase occurred only in the peripheral portion of the lobule and was never observed in the central zone where the number of binucleated cells remained stationary at an average of 10 per 100 liver cells. Hence these data have not been put in the table. Control animals which received either no medication or plain sesame and Wesson oils did not show a rise in the number of binucleated cells. Likewise the rabbits which received thyroxin failed to show this change, indicating that even if the basal metabolism of the animals was elevated, it did not cause the increase in binucleated cells.

In order to determine whether or not vitamin D was the specific cause of this phenomenon, the effect of inactivated viosterol was studied. Viosterol was exposed for 98 hours to a mercury vapor quartz light at a distance of one foot. This over-irradiated viosterol was tested in rachitic rats in the usual manner and found to be completely inactivated. When the inactivated viosterol was administered to rabbits by stomach tube, in doses of 3 cc, the same result was obtained as when potent viosterol was used. Because this was an unexpected result and because viosterol is not a solution of pure vitamin D_2, the experiments were repeated, using Drisdol, which is a solution of crystalline vitamin D_2 in propylene glycol. Again the results were surprising. The daily administration of 3 cc of Drisdol, or a similar dose of Drisdol which had been inactivated by over-irradiation with ultraviolet light for 120 hours, as well as the solvent propylene glycol, led to a definite increase in the number of multinucleated cells in the peripheral zones of the lobules.

No correlation was found in any of the experiments between an increase in organic phosphates in the blood serum and an increase in binucleated cells in the peripheral zone of the lobule. For example, hyperphosphatemia occurred in rats and dogs fed viosterol, but the binucleated cells did not increase. On the contrary, the administration of inactivated viosterol and of Drisdol did not cause hyperphosphatemia in rabbits, but did result in an increase in the number of binucleated cells. These results proved beyond doubt that the phenomenon was not caused by vitamin D itself.

Because vitamin D, inactivated vitamin D and propylene glycol are chemically alcohols, preliminary experiments were made by

TABLE I.

Animal	Amount of drug administered	Duration of experiment days	% of binucleated cells in periphery of liver lobule
Rabbit			
16	Viosterol 3 cc	4	27
17	” ”	6	65
18	” ”	8	58
6	” ”	8	43
33	” ”	12	53
7	” ”	14	45
25	” ”	15	58
37	Irrad. Viosterol 3 cc	6	40
31	” ” ”	12	61
1	Wesson Oil 3 cc	8	27
27	Thyroxin 0.2 mg	8	21
29	” 0.3 ”	8	20
28	” 0.3 ”	9	25
38	Drisdol 3 cc	8	49
39	” ”	15	73
42	Irrad. Drisdol 3 cc	8	54
43	” ” ”	10	21
15	Sesame Oil 3 cc	8	28
36	” ” ”	14	13
14	” ” ”	15	26
40	Propylene Glycol 3 cc	6	68
41	” ” ”	15	46
55	Ethyl Alcohol 10 cc	1	42
54	” ” 5 ”	4	22
60	” ” 10 ”	4	26
57	Ether 2 cc	1	44
56	” 1 ”	2	45
47	” 3 ”	1	26
48	” 3 ”	2	24
13	Control	15	25
50	”	15	23
51	”	15	20

feeding absolute alcohol and ethyl ether (Squibb) to rabbits. As seen in the table, both alcohol and ethyl ether caused an increase in the number of binucleated cells in the livers of some, but not all, of the animals treated. Thus it would appear that although these substances can, they do not constantly, bring about the increase, and further that the cellular change is not specific for alcohols.

Summary. It has been shown that the increase in binucleated cells in the peripheral zone of the lobule of the liver which Szittay observed after the feeding of Vigantol to young rabbits is not due to a specific action of vitamin D as he believed. The increase also occurs after the administration of inactivated viosterol, Drisdol, inactivated Drisdol, propylene glycol, absolute alcohol and ethyl ether.

The fact that young rats and dogs do not show this phenomenon, even though vitamin D is known to be just as effective against rickets in these animals as in rabbits, suggests that there is a species difference.

There is no detectable relationship between the effect of vitamin D on the phosphorus and calcium content of the blood and the increase of multinucleated cells in the liver. It is unlikely that an increase in basal metabolism is responsible for the phenomenon since the administration of thyroxin does not lead to an increase in the number of multinucleated cells. Wesson oil and sesame oil are without effect.

The observation that ethyl ether as well as propylene glycol and absolute alcohol may bring about an increase in the number of multinucleated cells suggests that a number of chemically different substances may be responsible for the increase.

13296 P

Hemorrhage Control in *Eimeria tenella* Infected Chicks When Protected by Anti-Hemorrhagic Factor, Vitamin K.

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Excessive amounts of vitamin K in the dietary are usually considered a necessary factor in cases where prothrombin levels must be built up. However, as far as the authors are aware there has been no work on the control of hemorrhage in cases of parasitism by the coccidium, *Eimeria tenella,* in chicks in relation to the antihemorrhagic vitamin K. This vitamin was obtained in the liquid form, a peanut oil compound, under the trade name, "Klotogen".* A pure culture of *E. tenella* was obtained from the Los Angeles

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* Through courtesy of Abbott and Company, Los Angeles, California. (Each cc of this liquid vitamin is equivalent to 1250 Almquist units, or 46,500 Dam units.)

Poultry Pathological Laboratory, but was sub-cultured through two preliminary groups of chicks in order to establish the purity and virility of this strain. Previous work in this laboratory had shown that an incubation period of 5 days is followed without exception by an interval of high mortality in pens where the inoculation by *E. tenella* has been heavy (5,000 to 6.000 oocysts).

Thirty one-week-old chicks were wing-banded and segregated into 3 groups of 10 each. The segregation was at random, the only thought being to have the total weight similar in each group. The cages were constructed so that all droppings fell through wire-mesh floor where they could be removed for examination.

The 3 groups were fed the same dietary of baby chick mash† consisting of: ground corn, wheat bran, wheat shorts, ground wheat, feeding oat meal, dehydrated alfalfa meal, sardine fish meal, meat and bone scraps, soya bean oil meal, old processed linseed meal, dried skim milk, kelp meal, ground limestone, steamed bone meal, charcoal, salt, yeast, and Napco XX (fortified cod liver oil). Group 1, the controls, were maintained on this diet for the duration of the experiment. Group 2 were given 6,000 oocysts of *E. tenella* each the first day, plus 0.25 cc of Klotogen for 4 successive days. Group 3 were given 6,000 oocysts of *E. tenella* each on the first day but no Klotogen.

Group 1, the controls, did not show any symptoms of infection from any source, and grew rapidly from day to day. The average body weight of this group at the outset of the experiment was 141.6 g, and reached a total of 230 g in 9 days. This was an average daily gain in weight of 10.54 g for each bird. All caeca examined in this group were normal on autopsy.

Group 2, the vitamin K protected group, first showed symptoms of infection late in the fifth day of 5 bloody droppings. Early in the sixth day, bird No. 114 died and 24 bloody droppings were recovered from the group. On the seventh day 24 bloody droppings were examined. On the eighth day all hemorrhage had ceased. This group averaged a daily gain of 5.58 g. On autopsy all caeca were near normal except that of the one that died (114) which contained thick clotted blood. The hemorrhagic cores of the remaining caeca were very small and in one instance was lacking altogether, giving evidence that resorption had taken place.

Group 3, the unprotected group, first showed symptoms of parasitism early in the fifth day; 33 bloody droppings with a much greater amount of blood in each dropping than showed in those of Group 2 at the same time. The mortality began early in the fifth day with 7 birds dying during this time. On the sixth day there were 26 bloody droppings but no mortality, and on the seventh day there were 26 bloody droppings which were the last indications of hemorrhage. All the blood in all the droppings from this group was very fluid and watery. The 3 survivors never were very ill since they gained in weight rapidly after the fifth day.

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† California Milling Corp. Baby Chick Mash.

Conclusion. 1. Administration of vitamin K in the dietary of chicks definitely decreased the mortality in chicks parasitized by *E. tenella,* a hemorrhage-producing protozoan, since all autopsies performed show the vitamin K-protected birds had a fibrinated core in the caeca, which was in the process of being reabsorbed. 2. Vitamin K-protected birds showed a mortality of only 10% in comparison to 70% mortality in the unprotected group.

13297

Influence of Body Size on Gaseous Nitrogen Elimination During High Oxygen Breathing.*

ANDRE COURNAND, IRVING G. YARMUSH AND RICHARD L. RILEY.
(Introduced by Walter W. Palmer.)

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By breathing a gas mixture whose nitrogen content is low, as by the inhalation of almost pure oxygen, the nitrogen physically dissolved in body fluids and tissues is gradually excreted in the expired air. A correction for excreted nitrogen is important in the determination of the residual air of the lungs according to certain methods, such as that of Christie[1] or the more recent open-circuit method of Darling, Cournand, and Richards.[2]

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[1] Almquist, H. S., and Stokstad, E. R., *Nature,* 1935, **136**, 31.

[2] Becker, E. R., *Coccidia and Coccidiosis of Domesticated Game and Laboratory Animals and of Man,* 1934.

[3] Dam, H., and Schonheyder, T., *Biochem. J.,* 1934, **28**, 1355.

[4] Dam, H., *Biochem. J.,* 1935, **29**, 1273.

* Under a grant from the Commonwealth Fund.

[1] Christie, R. V., *J. Clin. Invest.,* 1932, **11**, 1099.

[2] Darling, R. C., Cournand, A., and Richards, D. W., Jr., *J. Clin. Invest.,* 1940, **19**, 609.

The rate of excretion of nitrogen during short periods of oxygen breathing has been studied by Campbell and Hill,[3] Behnke, Thomson, and Shaw,[4] and Darling, Cournand, Mansfield, and Richards.[5] As reviewed by the latter investigators, the values in the literature are in general agreement, averaging approximately 220 cc of nitrogen excreted during 7 minutes of pure oxygen breathing. An examination of the individuals upon whom nitrogen excretion values were determined, however, reveals that all were adults and of fairly large size. If the open-circuit method is to be applied to smaller individuals and children, a more accurate correction for nitrogen excretion must be obtained, since the correction becomes more important, the smaller the residual air.

The purpose of this study was to determine nitrogen excretion values in subjects of various ages and sizes. From this, it was hoped that a regression curve and formula could be established that would enable one to obtain a correction factor depending on the size of the individual.

Experimental. An effort was made to obtain as large a range as possible, in size and age of individuals. Since, however, other authors had made determinations on adults, we emphasized smaller individuals and children. Table I gives the age and physical characteristics of a group of 30 subjects on whom determinations were made. Of these, 23 were under the age of 20, while several were under 10.

TABLE I.

	Mean	Standard deviation	Range
Age (years)	17.8	± 9.6	9 - 44
Weight (kg)	54.8	± 18.0	27 - 93
Height (cm)	161.2	± 16.7	129 -188
Surface Area (sq.m)	1.56	± 0.34	0.98- 2.15

All subjects were under basal conditions with at least 6 hours of fasting and a period of rest of 30 minutes. The residual air was first determined by the method of Darling, *et al.*,[2] after which at least 15 minutes were allowed to elapse before the start of the nitrogen excretion determination. The latter was carried out in duplicate, with 15 minutes or more between the first and second runs, using the method of Darling, Cournand, Mansfield, and Richards.[5] The essential features of this method are: (a) a series of 4 maximally deep breaths of pure oxygen, by which the nitrogen of the intrapulmonary air is reduced to approximately 4%; followed by (b) a 7-minute period of breathing pure oxygen, the expired air of this period being collected and analyzed for nitrogen. If the residual air volume of the lungs is known, the excess nitrogen excreted from the body during this interval can readily be calculated.

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[3] Campbell, J. A., and Hill, L., *J. Physiol.*, 1931, **71**, 309.

[4] Behnke, A. R., Thomson, R. M., and Shaw, L. A., *Am. J. Physiol.*, 1935, **114**, 137.

[5] Darling, R. C., Cournand, A., Mansfield, J. S., and Richards, D. W., Jr., *J. Clin. Invest.*, 1940, **19**, 591.

For the analysis of the high-oxygen samples, the Van Slyke manometric apparatus was used throughout.

Results. The values of nitrogen excretion have been plotted against body surface area and body weight. The former is shown in Fig. 1. The coefficients of correlation are:

Nitrogen excretion and Surface Area: r = +0.797, S.E. ± 0.067
Nitrogen excretion and Body Weight: r = +0.800, S.E. ± 0.066

The regression formulae calculated are:

Nitrogen excretion (cc) = 96.5 Body Surface Area (sq.M) +35, S.E. ±25
Nitrogen excretion (cc) = 1.82 Body Weight (kg) + 76, S.E. ± 25

Discussion. The method for the determination of nitrogen excre-

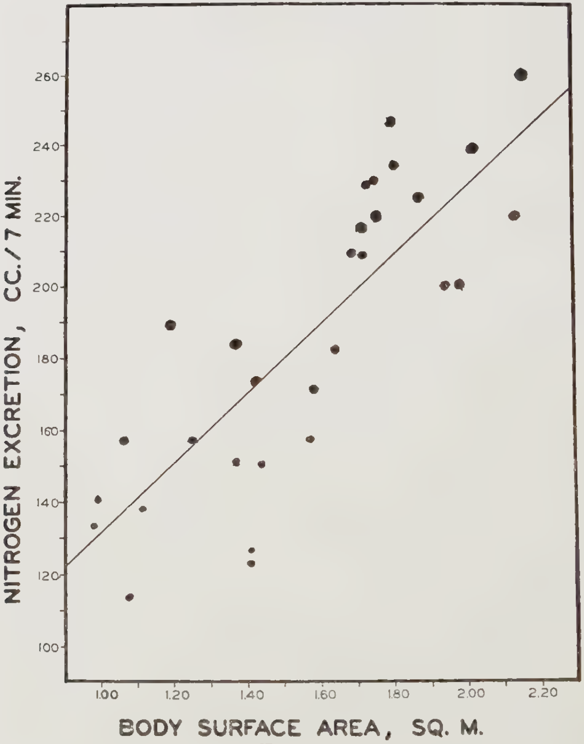

FIG. 1.

tion which was used here,[5] proved to be fairly precise. Out of 30 subjects on whom nitrogen excretion was determined, 18 were done in duplicate. The greatest difference among duplicates amounted to 56 cc and only 5 showed a difference greater than 25 cc.

In all of our experiments, the average alveolar nitrogen concentration, after the preliminary washing period, was 3.51% with a standard deviation of ±2.11 and a range from 1.30% to 9.51%. The nitrogen washed out subsequently, therefore, came mostly from the blood and tissues and only in very small amounts from the residual air. This fact undoubtedly helps to explain the fairly high precision of the method.

The maximum error in the determination of residual air by the open-circuit method, due to the calculation of nitrogen excretion from our regression formulae, would be 95 cc $\left(\frac{3 \text{ x } 25}{0.8}\right)$. In 68% of the cases it would not exceed 30 cc $\left(\frac{25}{0.8}\right)$.

In Fig. 2 the regression line (solid) of nitrogen excretion against body surface area has been drawn with lines (broken) parallel to it

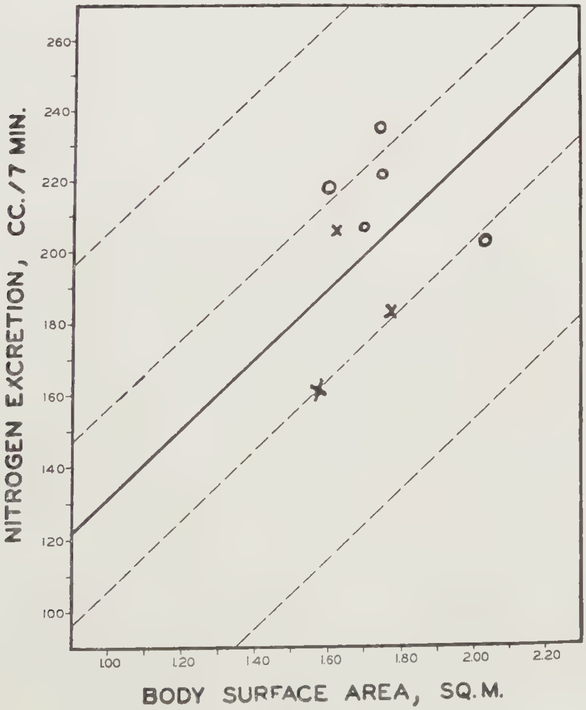

FIG. 2.

representing the limits of the standard error of our equation and 3 times the standard error. The values for nitrogen excretion during a 7-minute period of pure oxygen breathing calculated from the data of Campbell and Hill[3] (open circles) and Behnke, Thomson and Shaw[4] (crosses) have been plotted. As can be seen, 62% (5/8) of the points fall within the limits of the standard error while the rest fall easily within 3 times the standard error.

Summary. 1. The volume of nitrogen excreted from the body by way of the lungs, when a subject breathes pure oxygen for short periods, is found to vary with the size of the individual. 2. The nitrogen excretion during 7 minutes of oxygen breathing has been determined in 30 normal individuals of various ages and sizes. The values obtained have been correlated with body weight and with surface area. Coefficients of correlation and regression equations are given.

13298 P

Electromyographic Studies on Recoördination of Leg Movements in Poliomyelitis Patients With Transposed Tendons.*

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Man, in contrast to lower mammals,[1] can retime the action phase of a transposed muscle in accordance with its new function. Just how this occurs, is largely unknown. A systematic investigation of "recoördination," therefore, offers points of great theoretical and practical interest. A first report of our results is herewith presented.

Technic. The muscular action potentials were amplified in an ordinary vacuum tube amplifier set and recorded by an electromagnetically driven stylus writing on "Teledeltos" paper. Before entering the recorder, the amplified action currents passed through rectifying and integrating sets, partially summating and integrating individual spikes so as to give an estimate of the intensity of the contraction. Three identical channels were in operation, admitting independent simultaneous recording from three muscles. Provisions were made for the synchronization of these records with motion pictures. The electrodes consisted of copper mesh embedded in moist agar pads and strapped to the skin, cca 1 inch apart. Needle electrodes inserted through the skin did not prove significantly superior to surface leads. Elaborate precautions and checks were devised to guard against leakage of current from other than immediately subjacent muscles.

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* These investigations were aided by a grant from the National Foundation for Infantile Paralysis, Inc.

† With the invaluable and most gratifying coöperation of Dr. C. Howard Hatcher, Department of Surgery, and Mrs. Margaret C. Winters, Physiotherapy.

[1] Rat—R. W. Sperry, *J. Comp. Neur.*, 1940, **73**, 379. For full review of the problem, see P. Weiss, *Comp. Psychol. Monogr.*, 1941, **17**, No. 4.

Tests. For the sake of standardization, the tests were restricted to a single type of operation, namely, transposition of the tendon of the M. biceps femoris to the extensor side of the knee joint to substitute for the weakened or lost action of a paralyzed M. quadriceps. The action of the transplant was to be compared with its preoperative flexor and extensor relations. The muscles chosen to represent the knee extensor and flexor group were the residual M. rectus femoris and the inner hamstrings, respectively. The action phases of these muscles during a number of standardized test performances (simple voluntary movements on command, resisted and unresisted; from prone, supine, seated, standing position; stretch reflexes; walking; bicycling; stooping, etc) were determined in both normal and poliomyelitic (unoperated) individuals. With these data as background, the behavior of the transplanted M. biceps could be systematically followed.

Results. Twenty cases of biceps transplantation (18 poliomyelitis, 2 spastics) have thus far been explored. Six of them were available for study both before and after the operation, while the others were old cases operated on from 2-10 years previously. The following observations are condensed from cca. 2500 feet of records.

Pre-operative. The electromyogram has revealed appreciable residual activity in many paralyzed muscles rated as negative by palpation. Steady activity of the weak M. quadriceps during flexor as well as extensor phases seems to be characteristic of coördination in poliomyelitics (except in side-lying position), in contrast to normal and spastic individuals. The M. biceps operates in flexor phase only.

Postoperative. Records taken when the operated leg was removed from the cast for the first time, showed no activity of the transplant during the early efforts of the patient to move. Soon, however, either during the first or one of the succeeding sessions, the transplant began to come in, at first in flexor phase. After that, only surprisingly few trials were required to make the transplant suddenly con-

FIGS. 1 AND 2.

Electromyograms of thigh muscles during voluntary extension (E) and flexion (F) of knee. In all records the tracings, read from top to bottom, represent (1) M. biceps femoris, (2) Inner hamstring muscles, (3) M. rectus femoris, (4) Time signal in seconds.

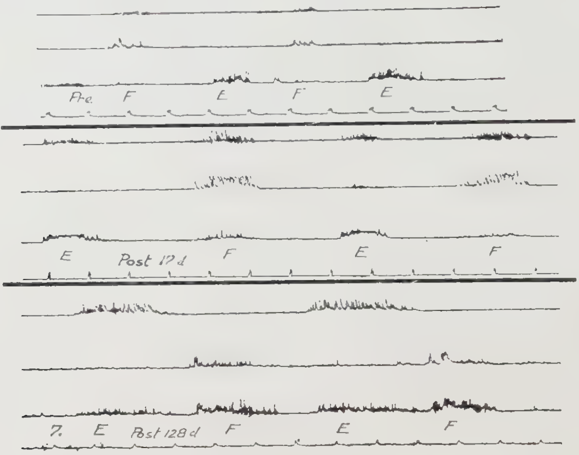

Fig. 1.

Three records taken from the same patient at different times. *Top:* Prior to the operation (side-lying position). Note Biceps action in flexor phase only. *Middle:* 17 days after tendon transposition. Note Biceps action in both flexor and extensor phases (F, E). *Bottom:* 128 days after the operation. Note absence of Biceps action during flexor phase.

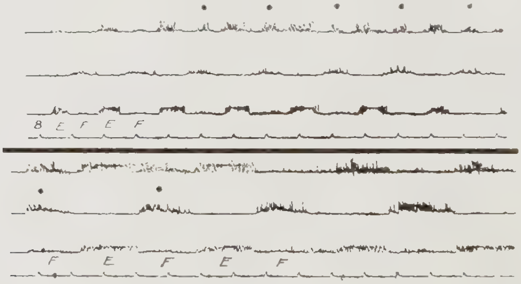

Fig. 2.

Relapse of transplanted Biceps into original flexor phase. *Top:* During fatigue towards end of prolonged test. Dots indicate occurrence of relapse. *Bottom:* Disorientation at beginning of performance. After two initial errors (marked by dots) transplant drops again its flexor association.

tract in extensor phase, too. Visualization of the task to extend the leg seems to be the prime aid to the patient; actual visual control and proprioceptive cues seem to be less important during the early phase of recovery. The transplant continues for some time to act in both flexor and extensor prases, and there is no evidence of automatic resumption of reciprocal innervation. Association of the biceps with the extensors does not by itself produce dissociation from the flexors. Only after a further practice period of individually varying duration, does the transplant begin to be omitted during flexor actions.

Even then, however, temporary relapses into the old flexor association occur repeatedly, even years after the operation. These relapses seem to be favored by fatigue, lack of concentration, automaticity of movement, etc. Their occurrence supports the view that the adjusted use of the transplant is not based on the substitution of a permanent extensor association for its former flexor association in the elementary motor mechanisms, but rather on the development in higher centers of a new type of action which can effectively override the innate coördinative associations without abolishing them. This corroborates the distinction between lower, rigid, and higher, plastic systems in the control of coördination suggested by earlier observations.[2]

These and numerous other facts still under examination (the fate of stretch reflexes; action of motor units; differential fatigue) exemplify the advancement of theoretical insight and practical knowledge concerning coördination which physiologists and orthopedic surgeons alike may expect to result from the electromyographic study of transplanted muscles in man.

13299

Protection in White Mice With Human Convalescent Serum Against Infection With Poliomyelitis Virus (Armstrong Strain)*†

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The results reported in this communication are the outcome of an attempt to reëvaluate the *in vivo* action of human convalescent serum, utilizing Swiss mice infected with the Armstrong strain of poliomyelitis virus.

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[2] Weiss, P., and Ruch, T. C., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 569.

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

† The human convalescent serum used in these experiments was obtained from patients of the Walnut Street School for Crippled Children, in Lansing, Michigan, and of the Blodgett Convalescent Home, in Grand Rapids, Michigan. To both the donors and to the staffs of these institutions and the many others who made the collection of the blood possible the author wishes to express his deepest appreciation.

At the moment human convalescent serum is in disrepute as a therapeutic or prophylactic agent. There is little to be gained in reviewing the many pros[1] and cons[2] regarding its effectiveness since it was first used in the treatment of the human disease in 1911.[3] It might perhaps suffice to say that its use appeared rational, since human convalescent serum possesses the property of neutralizing potent suspensions of monkey adapted virus.

Attempts to establish the usefulness of convalescent serum as a prophylactic and therapeutic agent in the experimental disease in the monkey have been equally inconclusive. The most complete study testing its prophylactic value was carried out by Schultz and Gebhardt.[4] These authors reported suggestive protection in monkeys against small infective doses of virus following the administration of large doses of highly potent immune horse and convalescent human and monkey serums. Our own continued attempts[5] to modify the disease in the monkey by the administration of large doses of human convalescent serum (10-20 cc per pound body weight) following infection, have been negative. The infectivity of the cords of those treated animals likewise appeared unmodified.

It has long been recognized, however, that there have been serious limitations to the use of the monkey as the experimental animal. The successful transmission by Dr. Charles Armstrong of the National Institute of Health[6] of a monkey strain of poliomyelitis virus to the cotton rat and white mouse has supplied an effective tool.

Experience with the Armstrong strain during the past 20 months, which has included a clinical and pathological study of this strain in monkeys, as well as in rats and mice, and a comparison by means of cross protection tests with at least 3 other strains of virus, leads us to concur with others[7] in the belief that this is a strain of poliomyelitis virus.

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[1] *a.* Aycock, W. L., and Luther, E. H., *J. A. M. A.*, 1928, **91**, 387; *b.* Aycock, W. L., Luther, E. H., McKhann, C. F., Smith, E. C., and Kramer, S. D., *J. Infect. Dis.*, 1929, **45**, 175; *c.* Levinson, S. O., McDougall, C., and Thalhimer, W., *J. A. M. A.*, 1932, **99**, 1058; *d.* McKenzie, M., *Canad. M. A. J.*, 1929, **21**, 291; *e.* Brebner, W. B., Preliminary Report of the Results of the Administration of Normal Adult Serum in the Prophylaxis of Poliomyelitis During the 1932 Epidemic, Poliomyelitis, Williams & Wilkins Co., 1932, p. 529; *f.* Stokes, J., Jr., Wolman, I. J., Carpenter, H. C., and Margolis, J., *Am. J. Dis. Child.*, 1935, **50**, 581.

[2] *a.* Kramer, S. D., Aycock, W. L., Solomon, C. I., and Thenebe, C. L., *New Eng. J. Med.*, 1932, **206**, 432; *b.* Park, W. H., *J. A. M. A.*, 1932, **99**, 1050.

[3] Netter, A., Gendron, and Touraine, *Compt. rend. Soc. de biol.*, 1911, **70**, 625, 707, 739.

[4] Schultz, E. W., and Gebhardt, L. P., *J. Ped.*, 1935, **7**, 332.

[5] Kramer, S. D., unpublished.

[6] *a.* Armstrong, C., *Pub. Health Rep.*, 1939, **54**, 1719; *b.* Armstrong, C., *Pub. Health Rep.*, 1939, **54**, 2302.

Experimental. Strain of Virus and Virus Dilutions. The strain of virus kindly supplied to us by Dr. Charles Armstrong was employed throughout these experiments. Since August, 1940, this strain has remained quite constant in potency. Three 10-fold dilutions of the virus were employed (5%, 0.5%, and 0.05%) in all but Experiments XVI and XVII.

Route of Infection. Infection was accomplished by the intracerebral inoculation of 0.03 cc of the virus suspension into the left cortex.

Incubation Period. This has varied with the concentration of infecting dose employed. In general the correlation between dose of virus and incubation period has followed the pattern described by Sven Gard, as it applies to infection with viruses by the intracerebral route.[8]

Animals. Three-week-old Swiss mice averaging 10 g in weight were employed throughout the experiments. Eight mice were used for each dilution (except in Experiments XVI and XVII). When a mouse died within a few hours following inoculation, it was subtracted from the total number.

Controls. Comparable numbers of control animals were simultaneously infected in each experiment. The control animals received either no serum or normal monkey serum (see charts).

Source of Serum and Method of Administration. The serum used in all but Experiment I came from a single pool of 13 donors. All donors had had the disease within 2 years and showed residual paralysis. A neutralization test was done on each serum and those possessing good neutralizing power were pooled. The pools were filtered, tested for sterility, and bottled without preservative.

The serum (human convalescent and normal monkey) was administered by the intraperitoneal route. Details of the administration of the serum with regard to amount, frequency, and time in relation to infection are indicated in each of the experiments in Charts 1 to 4 inclusive.

Two additional experiments (not illustrated in the charts) were

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[7] *a.* Rivers, T. M., *Infantile Paralysis, 1941,* published by the National Foundation for Infantile Paralysis, Inc., p. 60; *b.* Harford, C. G., and Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 211.

[8] Gard, S., *J. Exp. Med.,* 1940, **72**, 69.

CHARTS 1-4.

In Vivo Protection in White Mice with Human Convalescent Serum Administered Intraperitoneally Against Intracerebral Infection with Poliomyelitis Virus (Armstrong Strain).

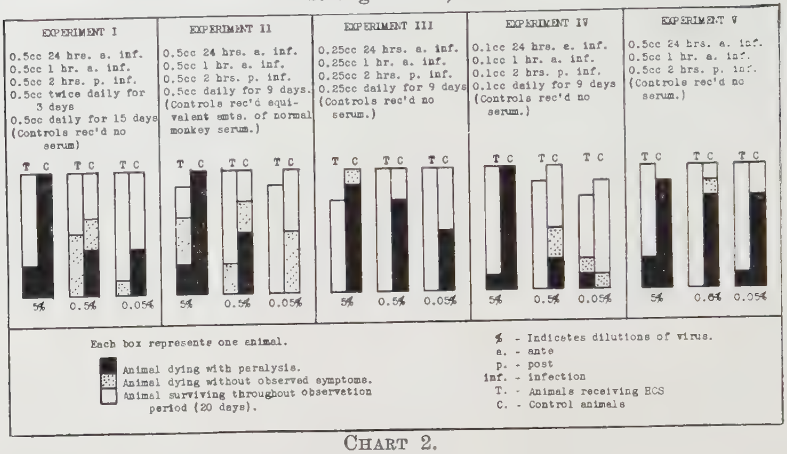


CHART 2.

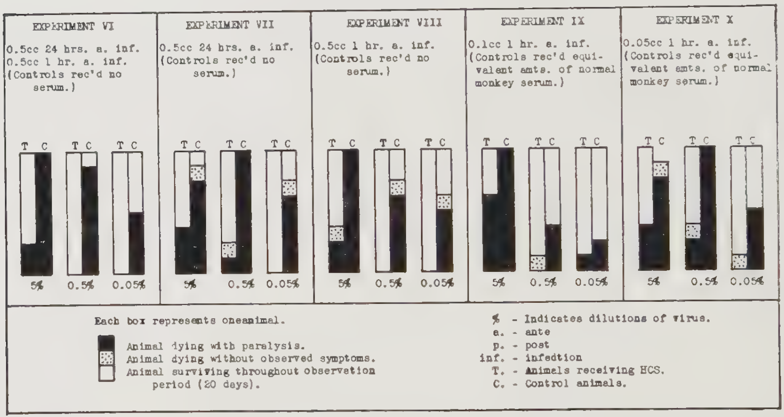


CHART 3.

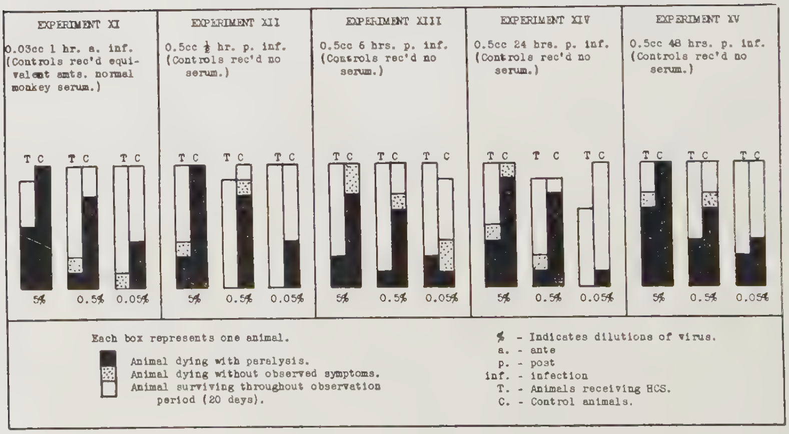
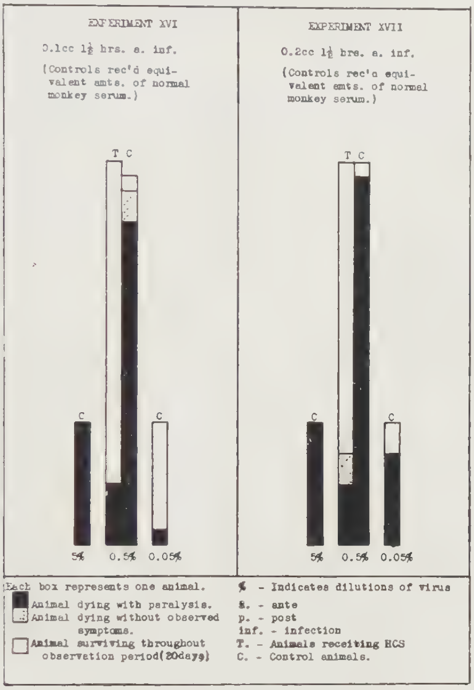


CHART 4.



conducted, in which immune globulin‡ was used in place of human convalescent serum. The dosage, method and frequency of administration were essentially the same as used in Experiment I.

Results. From the results of Experiments I and II, two things of possible significance stand out. When 0.5 cc of human convalescent serum is administered with the frequency and time with relation to infection indicated in the chart, significant protection is apparent in both experiments. This is also apparent in Experiments III and IV, in which the dose of serum administered was reduced to 0.25 and 0.1 cc respectively. Comparable though not as striking protection was obtained in the 2 experiments in which immune human globulin was administered in place of convalescent serum. The second point of significance (not illustrated in these charts) was the failure of the continued use of serum or immune globulin to modify the illness. Animals which contracted the disease did so within the normal incubation period and these animals lived no longer than did control animals developing the disease; in both groups, treated and control, such animals died from one to 5 days after the onset of symptoms.

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‡ Kindly supplied by Lederle Laboratories.

In Experiments V, VI, VII, and VIII, the individual dose of serum was maintained at 0.5 cc but the total quantity administered and the time with relation to infection was varied. Protection is apparent in all 4 experiments, even when a single dose of 0.5 cc was administered 24 hours before infection.

In Experiments IX, X, XI, single doses of convalescent serum but in varying amounts were administered to the treated group of animals one hour before infection. The results indicate that appreciable protection resulted even when 0.03 cc of serum was administered; although protection was not as apparent as when 0.5 cc of serum was employed (Experiment VIII).

In Experiments XVI and XVII (Chart 4), 0.1 cc and 0.2 cc respectively of convalescent serum was administered 1½ hours before infection. The more critical middle dilution was selected as the infecting dose of virus. Twenty-five mice were placed in each of these groups with comparable numbers of controls. A smaller group of control untreated mice (8 mice for each of the other 2 dilutions) was included as a check on the titration of the virus. The results appear clearcut; significant protection was obtained in the animals receiving the convalescent serum over the controls which had received equivalent amounts of normal monkey serum.

In Experiments XII, XIII, XIV, and XV (Chart 3), an effort was made to determine whether or not protection could be obtained if the convalescent serum was administered after infection. Significant protection was obtained when the serum was administered ½, 6 and 24 hours, but not 48 hours, after infection.

Summary. This report is based on 19 completed experiments designed to reëvaluate the usefulness of human convalescent serum.

From these experiments it would appear that it is possible to protect a significant number of 3-week, 10 g mice against intracerebral inoculation of poliomyelitis virus (Armstrong strain) by the intraperitoneal administration of from 0.03 to 0.5 cc of potent human convalescent serum—protection being somewhat more evident in those mice receiving larger doses of serum. Evidence of protection is furthermore obtained when the serum is administered up to 24 but not 48 hours after intracerebral infection.

Under the experimental conditions there appears to be no evidence that convalescent serum had any therapeutic effect, even when this was administered continuously from 24 hours prior to infection to 9-15 days following infection. Animals that succumbed to the disease did so within the usual incubation period and died, as did the controls, from one to five days after the onset of paralysis.

No attempt is or can be made at this time to translate the results of these experiments in terms appropriate for human application. They are reported, however, because we believe they represent the first definite evidence of significant protection with human convalescent serum under the experimental conditions.

13300

Electrical Rectification in Single Nerve Fibers.

RITA GUTTMAN AND KENNETH S. COLE. (Introduced by H. B. Williams.)

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The electrical resistance of an ordinary conductor is independent of the magnitude or direction of a current sent through it, and a current may pass with equal ease in either direction. There have been suggestions, however, that in a nerve fiber membrane, current may pass more easily in one direction than in the other, or that the nerve fiber membrane behaves as a rectifier rather than a simple resistance.

Recently, Cole and Baker,[1] and Cole and Curtis[2] demonstrated rectification in the squid axon, using alternating current bridge methods and the needle electrode technic. Another paper, Cole,[3] shows that the previous failure of Cole and Hodgkin[4] to observe rectification in this axon was due to the fact that only small currents had been used and to the fact that the effect under the anode was approximately equal and opposite to the effect under the cathode, thus neutralizing it.

By killing one end of the nerve fiber, neutralization of rectification under one electrode by that under the other can be avoided, and it should be possible to observe rectification directly by means of a much simpler technic involving a direct current Wheatstone bridge.

The giant nerve fiber of the hindmost stellar nerve of the squid, *Loligo pealii,* was used throughout the experiments. It was carefully dissected away from the smaller nerve fibers under sea water with the aid of a binocular microscope. Immediately after dissection the axon was threaded through a glass U tube filled with oil. Then the U tube was suspended by means of a glass rod cemented to it in such a way that one end of the fiber dipped into sea water, where it remained in good condition for many hours, and the other end into KCl isosmotic with sea water (0.52 M), which injured that end. The interelectrode region was about one cm and lay in oil. In some experiments a test solution, such as cocaine, was substituted for the sea water.

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[1] Cole, K. S., and Baker, R. F., *J. Gen. Physiol.*, 1941, **24**, 535.

[2] Cole, K. S., and Curtis, H. J., *J. Gen. Physiol.*, 1941, **24**, 551.

[3] Cole, K. S., *J. Gen. Physiol.*, 1941, **25**, 29.

[4] Cole, K. S., and Hodgkin, A. L., *J. Gen. Physiol.*, 1939, **22**, 671.

Two calomel electrodes lead to the measuring circuit, which consisted essentially of a direct current Wheatstone bridge (Fig. 1). The resting potential of the fiber was first balanced out by a potentiometer, P_2, using the bridge galvanometer, G. Then the variable resistance, R_3, was varied until balance was obtained in a galvanometer, G, used as a null point instrument, when a potential was applied to the bridge by means of a Leeds and Northrup student type potentiometer, P_1. The circuit included a switch, S, for reversing the direction of the current sent through the fiber.

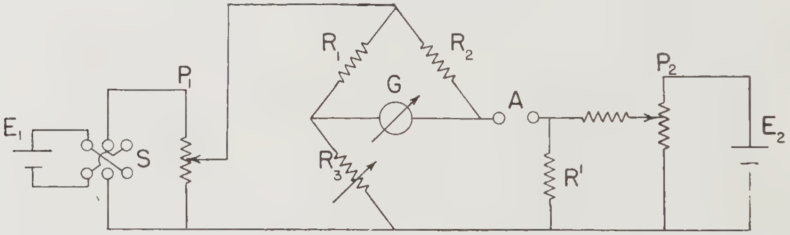

FIG. 1.

Measuring circuit, consisting of a D.C. Wheatstone bridge with ratio arms: R_1, 2000 ohms, and R_2, 5000 ohms; variable resistor, R_3, and galvanometer, G. E_1 and E_2 are 1.5 V. dry cells; S is a reversing switch; P_1, a student-type L. and N. potentiometer; P_2, a slide wire potentiometer; R^1, a 100 ohm resistor. A represents the axon.

A reading was made in the following way. After the potentiometer, P_1, had been set at the desired value, the reversing switch, S, was closed for an instant on one side. If a movement of the galvanometer needle occurred, showing that the bridge was off balance, the variable resistance, R_3, was reset, the amount of change necessary being estimated from the extent of the galvanometer needle swing. When balance was obtained the switch, S, was closed momentarily on the other side and a similar reading obtained for a current flowing in the opposite direction. Polarization of the fiber was minimized by this reversal of the current. During a run there were frequent returns to a reference E.M.F. value so that any shifting of values with time during the run could be compensated for in the calculations. Every effort was made to send currents in for short times only and to allow relatively long and equal intervals between readings so that the condition of the fiber did not change materially even though the currents used were often considerably over rheobase. (Under these conditions the rheobase was in the range 1-10 μ amperes.) With such treatment a fiber usually lasted many hours, but each run required about 20 minutes.

Experiments were done on 31 single nerve fibers. The figures represent typical results. It was possible to balance the resting potential to within 0.1 mv. With the lowest currents sent through the fiber the bridge could be balanced to within 25 ohms, which was usually about 0.1%. With larger currents the precision was correspondingly higher.

The resistance of the fiber was determined as a function of impressed E.M.F.'s and the data are plotted as resistances vs. currents, Figs. 2 and 3.

When the fiber is completely dead, it follows Ohm's Law, and resistance is independent of current, Fig. 2D. When the fiber is alive, it does not follow Ohm's Law, and the resistance depends upon the current, Fig. 2A, 2B, and 2C. This means that the live nerve fiber is a rectifier and allows current to pass more easily outward than inward. As the nerve fiber dies, the fiber shows less and less rectification.

It was possible to correlate rectification with resting potential changes and excitability by means of simultaneous readings on the same fiber, Fig. 2. As time elapses, the resting potential of a fiber approaches zero. Excitability was measured by recording action potentials with a cathode ray oscillograph set-up. In Fig. 2A and 2B, the nerve fiber was excitable. In Fig. 2C there was evidence of local response, but no propagated impulse. In Fig. 2D, not even a local response was evident and there is no rectification.

Rectification also decreases when the axon is anesthetized, and this loss is partially reversible, Fig. 3. In Fig. 3A, the axon was in sea water with a good resting potential present. When 1.5% cocaine · HCl in sea water immersed the end which was formerly in pure sea water, the resting potential dropped and rectification decreased, Fig. 3B. When the experimental end was returned to sea water, the resting potential rose and rectification returned somewhat, Fig. 3C. When the experimental end was again treated with cocaine, Fig. 3D, rectification again decreased, but this was irreversible, Fig. 3E, since these runs were all made on the same axon

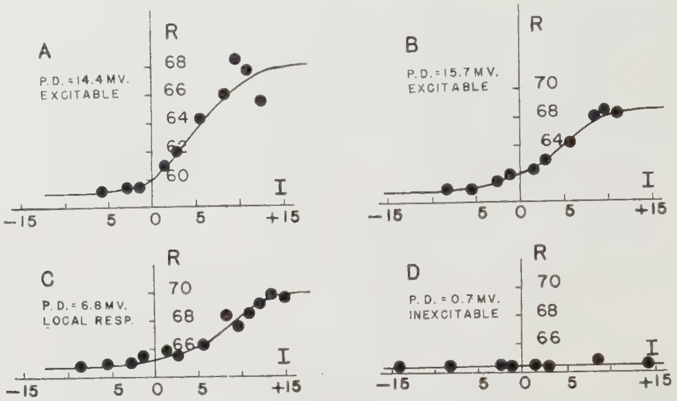

Fig. 2.

Loss of rectification with time in the squid giant nerve fiber. Time after completion of dissection: A, 6 min; B, ¾ hr; C, 2½ hr; D, 6½ hr. Current in microamperes, I, *vs.* resistance in thousands of ohms, R. Positive currents indicate that positive electrode is on uninjured portion of the nerve fiber. Resting potentials and excitability are noted for each run.

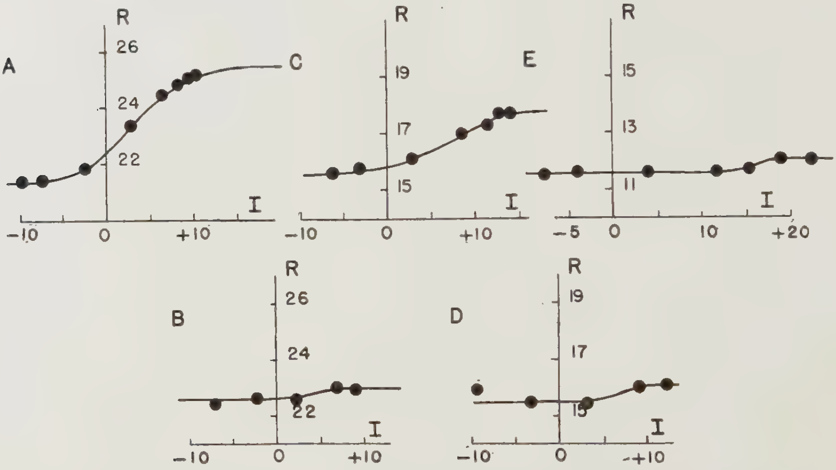

Fig. 3.

Partially reversible loss of rectification in the squid giant nerve fiber upon narcotization with cocaine • HCl in sea water. Time after completion of dissection: A, 1 hr; B, 1½ hr; C, 1¾ hr; D, 2¼ hr; E, 2½ hr. Current in microamperes, I, *vs.* resistance in thousands of ohms, R. Positive currents indicate that positive electrode is on uninjured portion of the nerve fiber. A, experimental end of fiber in sea water, P.D. = 30.0 mv.; B, experimental end in 1.5% cocaine, P.D. = 13.9 mv.; C, experimental end in sea water, P.D. = 27.4 mv.; D, experimental end in 1% cocaine, P.D. = 13.2 mv.; E, experimental end in sea water, P.D. = 12.8 mv.

and lasted a number of hours. After such prolonged treatment the fiber was no longer able to recover. Similar results were obtained with veratrine sulphate.[5]

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[5] For the effect of narcotics upon the membrane resistance of muscle fibers, *cf.* Guttman, R., *J. Gen. Physiol.*, 1939, **22**, 567.

The measuring technic used in these experiments has certain advantages. It is simple and it permits one to use an axon immediately after dissection. The accuracy required is not difficult to obtain. On the other hand, runs take about 20 minutes, and the immediate problem is to speed up the measurements. Also, the procedure consisted in measuring the differences between parts of the fiber in each electrode chamber. No absolute values could thus be obtained since the condition of the part of the nerve fiber in the sea water chamber was always referred to that of the part of the fiber in the chamber containing isosmotic KCl.

The measurements show that rectification is lost when the nerve fiber becomes inexcitable, when it dies, or when it is anesthetized. Rectification thus seems to be associated with the normal functioning of nerve.

Membrane potential[1] and impedance[2] measurements indicate that the rectifier effect is situated in the membrane, and it seems reasonable to assume that membrane conductance is a measure of ion permeability. These measurements then indicate that there is an increased ion permeability under a cathode and a decreased ion permeability under an anode. Interpreting in a similar manner the results of Ebbecke[6] obtained on frog nerve, we find large permeability changes at both anode and cathode, superimposed upon which there was a small permeability difference between the anode and cathode regions. Blinks also studied rectification in the giant plant cell, *Valonia*.[7] Rectification is probably responsible for at least a part of the non-linear potentials obtained by Pumphrey, Schmitt and Young,[8] and termed a "physiological" response by them.

Rectification may assist in explaining various alternating current phenomena in nerve, such as the delay of excitation with alternating currents,[9] alternating current block,[10] and the time of rise of electrotonus being more rapid at the cathode than at the anode.[11] However, the explanation of rectification, itself, is not as yet available. It will probably also be an explanation of the mechanism of the ion permeability of the membrane.

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[6] Ebbecke, U., *Pflüger's Arch.*, 1922, **195**, 555.

[7] Blinks, L. R., *J. Gen. Physiol.*, 1930, **13**, 793.

[8] Pumphrey, R. J., Schmitt, O. H., and Young, J. Z., *J. Physiol.*, 1940, **98**, 47.

[9] Gildemeister, M., *Ber. ü. d. Verhandl. d. sächs. Akad. d. Wiss. Math. Phys. Klasse*, 1929, **81**, 287; 303.

[10] Wedensky, N. E., *Pflüger's Arch.*, 1903, **100**, 1; Rosenblueth, A., and Reboul, J., *Am. J. Physiol.*, 1939, **125**, 251.

[11] Bogue, J. Y., and Rosenberg, H., *J. Physiol.*, 1934, **82**, 353.

13301

Experimental Air-Borne Disease. Quantitative Inoculation by Inhalation of Influenza Virus.

W. F. Wells* and W. Henle.† (Introduced by Stuart Mudd.)

From the Laboratories for the Study of Air-borne Infection and the Department of Pediatrics,† University of Pennsylvania School of Medicine.*

Successful infection of animals with a virus recovered from influenza patients[1] laid the foundation for experimental study of the mode of transmission of this important respiratory disease. Intranasal instillation of influenza virus recovered from artificially infected air[2] proved air conveyance of the virus to be possible, and destruction of the air-suspended virus by ultraviolet irradiation raised hopes of environmental control of epidemic influenza by sanitary ventilation. Demonstration of air-borne influenzal infection of ferrets[3] and introduction of quantitative inhalation technics of inoculation[4] provided experimental means for study of air-borne influenza and air-borne superinfections which frequently complicate the pathologic and epidemiologic patterns.

The experiments to be presented have been conducted during the fall and winter of 1939-40 when other duties delayed the continuation of this study until a preliminary report seems desirable. Of the 25 experiments performed, employing some 1500 mice, protocols have been chosen to illustrate our interpretation of the observed phenomena. These results have been found reproducible in repeated tests, but the exploratory nature of the protocols did not adapt them to statistical summary.

Experimental. I. Air-borne Transmission of Influenza A Virus. Exposure of white mice to air which had been infected by droplet nuclei derived from a suspension of the virus of influenza A produces typical signs of the disease in these animals and causes death with sufficiently high concentration of the virus. All mice exposed 45

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* These laboratories are supported by a grant from The Commonwealth Fund to the University of Pennsylvania.

† A grant in aid of this study was made by the Board of Managers of the Children's Hospital of Philadelphia.

[1] Smith, W., Andrewes, C. H., and Laidlaw, P. P., *Lancet*, 1933, **2**, 66.

[2] Wells, W. F., and Brown, H. W., *Science*, 1936, **86**, 68; *Am. J. Hygiene*, 1936, **24**, 407.

[3] Trillat, A., and Beauvillain, A., *Compt. rend. Acad.*, 1936, **205**, 1186; *Rev. d'hyg.*, 1938, **60**, 104; Andrewes, C. H., and Glover, R. E., *Brit. J. Exp. Path., Hygiene*, 1941, **34**, Sec. B, 21.

[4] Wells, W. F., *Science*, 1940, **91**, 172; Wells, W. F., and Lurie, M. B., *Am. J.* 1941, **22**, 91.

TABLE I.
Air-borne Infection of White Mice with Virus of Influenza A and Its Relation to Concentration of Virus in the Air.
From Experiment 12, February 22, 1940.

Dilution of virus (WS-85)	Time of exposure in min.	g of suspension lost	Mouse No.									
			1	2	3	4	5	6	7	8	9	10
Undiluted	45	6.0	D6	D6	D6	D8	D8	D8	D8	D9	D9	D9
10^{-1}	45	5.5	D9	D10	D10	4	3	3	3	2	2	2
10^{-2}	45	5.0	D10	3	2	2	2	1	1	±	±	0
10^{-3}	45	6.0	2	0	0	0	0	0	0	0	0	0

Key to Tables: Mice dying before the 10th day were recorded according to day of death—D4, D5, etc. Surviving mice were sacrificed on the 10th or 12th day and the degree of lung lesions recorded. 1 signifies ¼ consolidated; 2 signifies ½ consolidated, etc.

minutes to air infected by atomizing 1 g of a 10% suspension of mouse lung (infected with PR-8[5] or WS[1] strain) in 50 cubic feet of air died within 6 to 9 days. (Table I.) If one-tenth of this amount was used for the spray, 30% of the mice succumbed and the remaining animals harbored severe lung lesions when autopsied on the tenth day. A dilution of 1:100 of the original suspension resulted in lesions of various degrees in most of the mice, and a 1:1000 dilution produced practically no lesions at all.

This gradation parallels experience with the intranasal inoculation of various dilutions of virus into anesthetized mice. The gross appearance and the microscopical sections of the lung lesions did not seem to differ from those following intranasal infection. Lesions could be prevented by the intranasal administration of a potent mouse anti-influenza A serum previous to the exposure to the virus[6] and the agent could be recovered from the lungs by subsequent passage to normal mice by the intranasal route. No doubt, therefore, influenza A virus has been successfully transmitted to white mice by the air-borne route.

Lesions produced by the air-borne route were, however, decidedly less severe than expected from calculation of the amount of air-borne virus inhaled, as compared with intranasal instillation of similar quantities under anesthesia. The influence of the anesthesia or the fluid medium (broth) on the severity of the lung lesion hardly accounted for the difference.

A few ferrets exposed to ferret-adapted virus showed a rise in temperature 48 hours later. Serum taken 2 weeks afterwards contained a high neutralizing titer for the virus of influenza A, while the serum taken before the start of the experiment was negative.

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[5] Francis, T., Jr., *Science*, 1934, **80**, 457.

[6] Henle, W., Stokes, J., Jr., and Shaw, D. R., *J. Immunol.*, 1941, **40**, 201.

II. Compound Infection with Influenza A Virus and Pneumococcus Type I. Although recent epidemics have shown that clinical manifestations of uncomplicated infections with the virus of influenza A are not severe in man, bacterial superinfections may entirely alter the pathologic and epidemiologic pattern of the disease. Compound infection with virus and bacterium is decisive in swine influenza,[7] but the relationship in human pathology is not yet clearly understood. Mixed infection of mice with human influenza virus and with hemolytic streptococcus of Lancefield's group C produced higher mortality than either agent alone,[8] and nasal infection of the ferret has been induced by the combined action of influenza A virus and a hemolytic streptococcus (Group C) differing from uncomplicated influenza.[9] Pneumococcus Type I seemed to be particularly fitted for a study of compound infection, for this organism unassisted has little virulence for white mice when administered by the intranasal[10] or the air-borne route.[11] Mortality from compound air-borne infection of mice with pneumococcus (Type I) and sublethal doses of hemolytic streptococcus (Group C) has been shown[4] to exceed the sum of mortalities from infection with either.

Mice infected by inhalation of air-borne influenza A virus were exposed 1 to 5 days later to air-borne pneumococci of Type I from a culture which proved fatal in 10^{-8} to 10^{-9} dilution when intraperitoneally injected. With large doses of virus (resulting in the death of all mice exposed), the pneumococcus, when given 24 hours after the virus, shortened survival times in a number of experimental animals. On the other hand, pneumococci inhaled as late as five days after exposure to sublethal suspensions of the virus increased the severity of lesions and death frequently ensued. Tables II and III illustrate these relationships.

All mice found dead as well as those sacrificed on the twelfth day were examined for the presence of pneumococci in the lungs and the heart's blood, positive findings being designated in the tables by italics. Gross lung lesions usually did not appear to differ from those due to the virus alone. Occasionally a mottled appearance contrasted with the more even brownish-red color of the influenzal lung lesion. Histological studies do not yet justify speculation regarding the specific pathologic changes introduced by superinfection with

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[7] Shope, R. E., *J. Exp. Med.*, 1931, **54**, 349.

[8] Schwab, J. L., Blubaugh, F. C., and Woolpert, O. C., *J. Bact.*, 1941, **41**, 59.

[9] Glover, R. E., *Brit. J. Exp. Path.*, 1941, **22**, 91.

[10] Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1933, **58**, 465.

[11] Stillman, E. G., *J. Exp. Med.*, 1933, **23**, 58.

TABLE II.
Effect of Pneumococcus Type I on Mice Infected with a Fatal Dose of Influenza A Virus.
From Experiment 16, March 14, 1940.

	Mouse No.									
Suspensions atomized	1	2	3	4	5	6	7	8	9	10
Virus alone (WS-88, 10% suspension)	D7	D7	D7	D8	D8	D8	D8	D10	D10	D10
Virus + pneumococcus 24 hours later	*D4*	*D5*	*D5*	D8	D8	D8	D8	D9	D9	D9
Pneumococcus alone	*D7	0	0	0	0	0	0	0	0	0

*No lung lesion.
Italics designate the isolation of the pneumococcus from the heart's blood.

TABLE III.
Effect of Pneumococcus Type I on Mice Infected with a Dilute Suspension of Influenza A Virus.
From Experiment 21, April 10, 1940.

	Mouse No.											
Suspensions atomized	1	2	3	4	5	6	7	8	9	10	11	12
Virus alone (WS-90, 10^{-2})	D11	D12	4	4	2	2	2	2	0	0	—	—
Virus + pneumococcus 5 days later	*D8*	*D8*	*D8*	*D8*	D9	*D10*	*D11*	*D11*	*D11*	D12	0	0
Pneumococcus alone	0	0	0	0	0	0	0	0	0	0	—	—

Italics designate the isolation of the pneumococcus from the heart's blood.

TABLE IV.
Influence of Ultraviolet Light Radiation on Air Infected with the Virus of Influenza A.
From Experiment 13, February 22, 1940.

		Mouse No.									
Suspension atomized	Ultraviolet radiation	1	2	3	4	5	6	7	8	9	10
WS-85 (10% suspension)	—	D6	D6	D6	D8	D8	D8	D8	D9	D9	D9
” ” ”	+	±	±	±	±	±	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0

the pneumococcus. These phenomena did not occur with inhaled sterile nuclei.

III. Sterilization of Infected Air by Ultraviolet Light. Radiant disinfection of air infected with fatal concentrations of influenza A virus (3 seconds exposure to an estimated 120 microwatts per square centimeter of 2537 Å wave length) is shown in Table IV. Only minor lesions were evident in the lungs of mice sacrificed on the tenth day after breathing irradiated air. Five out of 20 mice showed only one or two lesions of pinhead size. Comparison with Table I shows destruction of more than 99% of the virus.

13302 P

Influence of 2-4 Dinitrophenol on Liver Lipids of the White Mouse.

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The prominent rôle attributed to the liver in fat metabolism has led to the study of many substances which produce changes in the fat distribution and concentration in this organ. Although many drugs and poisons increase the liver total lipid, the liver phospholipid is usually a constant percentage of liver moist weight. An exception has been noted by Cahn[1] who found increases of 4 to 40% in the phospholipid of dog liver after lethal doses of 1-2-4 dinitrophenol. However, of all the substances studied, only thyroxine[2] has been found to produce an appreciable decrease in the liver phospholipid. Since dinitrophenol also has the property of increasing the metabolic rate,[3] it seemed reasonable that the chronic administration of this drug might also reduce the phospholipid content of the liver.

Methods. Mice in groups were given subcutaneously various doses of dinitrophenol for periods up to 7½ weeks (Table I). Standard lipid methods were used for the liver analyses.[5, 6, 7]

Results. Both the phospholipid and cholesterol percentages decreased; the phospholipid from 3.67%, the normal value, to 3.06%, the cholesterol from .35% to .25%. These decreases have been shown to have a high degree of statistical significance.[8] In 7 mice the neutral fat was decreased also; however, the average neutral fat values were not significantly different from normal.

Since the livers were of normal weight the phospholipid decrease indicated a real loss of phospholipid. Both thyroxine and dinitrophenol increase the metabolic rate and both decrease the phospholipid of the liver. This correlation points to the metabolic function of

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[1] Cahn, T., *Ann. Physiol. Physicochim. Biol.*, 1933, **9**, 393.

[2] Schmidt, L. H., *Am. J. Physiol.*, 1935, **111**, 138.

[3] Ronzoni, E., and Ehrenfest, E. J., *J. Biol. Chem.*, 1936, **115**, 749.

[4] Hodge, H. C., MacLachlan, P. L., Bloor, W. R., Stoneburg, C. A., Oleson, M. C., and Whitehead, R., *J. Biol. Chem.*, 1941, **139**, 897.

[5] Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

[6] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

[7] Kelsey, F. E., *J. Biol. Chem.*, 1939, **127**, 15.

[8] Fisher, R. A., *Statistical Methods for Research Workers*, 1938, 7th Ed., London.

TABLE I.
Average Data on Liver Lipids of Dinitrophenol Injected Mice.

				Liver		
Administration time	Liver moist wt, g	Liver per g mouse	Neutral fat % moist wt	Phospholipid % moist wt	Cholesterol % moist wt	Total lipid % moist wt
24 hr*	1.385	69.5	2.22	2.75	0.23	5.38
	1.359	64.6	2.21	2.75	0.23	5.21
	1.150	54.6		3.21		
Avg	1.298	62.9	2.21	2.86	0.23	5.30
96 hr	1.123	56.1	1.18	4.68	0.30	6.16
	1.041	52.1	1.00	3.24	0.27	4.52
	1.220	61.0	0.62	3.54	0.24	4.40
	.930	46.5	2.50	3.10	0.28	5.88
	1.077	53.8	1.28	3.38	0.21	4.87
Avg	1.078	53.9	1.12	3.59	0.26	5.16
6 wk†	1.647	68.7	0.86	2.81	0.26	3.93
	1.780	73.9	2.51	2.00	0.25	4.77
	1.623	67.7		2.89	0.25	
	1.612	67.4		2.81		
Avg	1.665	69.4	1.68	2.63	0.25	4.35
7 wk‡	1.045	41.9	5.31	2.93	0.30	8.54
	1.360	54.4	2.16	2.50	0.31	4.97
	1.490	59.6		2.89		
Avg	1.298	52.0	3.73	2.77	0.30	6.75
7½ wk§	1.857	74.3	1.13	2.95	0.09	4.28
	1.323	52.9	1.15	3.40	0.21	4.76
	1.429	57.2		3.19		
Avg	1.536	61.8	1.14	3.13	0.20	4.52
Avg treated	1.359	60.0	1.97	3.06	0.25	5.21
Std. Dev.				±.54	±.037	±.18
S. E. Mean				±.13	±.01	±.33
Avg normal	1.332	60.0	1.52	3.67	0.35	5.54
Std. Dev.				±.26	±.13	±.047
S. E. Mean				±.058	±.029	±.01

*High dosage, 37 ½ mg/kg body wt.
† ” ” 25 ” ” ”
‡Medium ” 10 ” ” ”
§Low ” 5 ” ” ”

phospholipid in the liver, previously established by Sinclair[9] and others.[10]

In mice given dinitrophenol, the heightened metabolic demands tend to remove rather than increase body lipids. The fact that with increasing metabolism the liver loses cholesterol emphasizes the view that cholesterol plays a rôle in the fat metabolism of the liver.

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[9] Sinclair, R. G., *J. Biol. Chem.*, 1932, **96**, 103.

[10] See bibliography of Sinclair, R. G., *Physiol. Rev.*, 1934, **14**, 351.

Summary. Under the stress of increased metabolic rate, due to dinitrophenol injection, liver phospholipid and cholesterol are decreased. These changes are consistent with the belief that at least part of the phospholipid and cholesterol of the liver are important in fat metabolism.

The authors wish to express their thanks to Dr. W. R. Bloor for his advice on this problem.

13303

Localization of Colloidal Dyes in Animal Tumors.

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Introduction. It has long been known that certain colloidal dyes when injected intravenously exhibit properties of selective accumulation in tumor tissue.* The possible use of such dyes or other similarly behaving colloids as "carriers" for localizing radioactive and slow neutron capturing materials in neoplastic tissue for internal radiation therapy was suggested recently by Zahl and Cooper.[3, 4] With the radiation properties of these materials in mind (that is, type of emitted particle, penetration path, ionization density, etc.), it seemed of value, in an effort to probe the usefulness of such a concept, to reëxamine the localization features of such "carriers" in tumor tissue. This seemed of particular importance in relation to (a) the gross distribution within the body and (b) the distribution pattern in the tumor mass.

The localization of certain dyes in tumors has been described in some detail by Ludford[5] and Duran-Reynals[1] on animal material, and grossly by Brunschwig, Schmitz and Clarke[2] on human material. Some of the points presented here are in general confirmatory of this earlier work, but are of renewed interest when considered from the standpoint of radiation possibilities.

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* For recent appraisal of this subject, *vid.*:[1,2]

[1] Duran-Reynals, F., *Am. J. Cancer*, 1939, **35**, 98.

[2] Brunschwig, A., Schmitz, R. L., and Clarke, T. Howard, *Arch. Path.*, 1940, **30**, 902.

[3] Zahl, Paul A., and Cooper, F. S., *Science*, 1941, **93**, 64.

[4] Zahl, Paul A., and Cooper, F. S., *Radiology*, 1941, in press.

[5] Ludford, R. J., *Proc. Roy. Soc. London*, Ser. B, 1929, **104**, 493.

Procedure. Although a number of newly prepared dyestuffs were tested as to gross tumor-localizing properties, representative detailed study was made only on the 2 well-known vital dyes, Evans Blue and Lithium Carmine.[†] The dyes were injected intravenously into somewhat more than 150 tumor-bearing mice in amounts of 0.1 to 0.5 cc of a 1.0-2.0% aqueous solution. Mice used were an inbred strain implanted with Sarcoma 180 or Mammary Carcinoma 15091a. Mice with spontaneous mammary carcinoma were also used.

Following injection of the dye solutions, the animals were sacrificed at varying intervals and their tissues studied histologically, with special reference to the tumor sites. Ordinary methods of fixation and dehydration do not remove the dyes. The usual procedure was to clear and mount one section unstained, stain the duplicate very slightly with hematoxylin so as not to mask the dye, and to stain the triplicate routinely for cellular detail. By comparing such sections, the exact location of the dye and roughly the quantity present could be easily ascertained.

No essential differences were noted in the behavior of Evans

[†] Other dyes tested for tumor localization properties were:

(1) Copper azo-dye (No. R-1124-25, Amer. Cyan.)—too acutely toxic for localization observations.

(2) Uranium azo-dye (No. R-1203-99, Amer. Cyan.)—decolorized soon after injection, preventing observation as to tinctorial localization.

(3) Lithium Pontamine Sky Blue 6B (No. L-2300, Dupont).

(4) Lithium Salt of Trypan Blue (Nat. Aniline).

(5) Lithium azo-dye (No. R-1209-68,18, Amer. Cyan.).

Dyes (3), (4) and (5) are probably isomeric or at least similar to Evans Blue (Eastman) and grossly similar in localizing properties.

(6) Pontacyl Fast Blue Conc. 5R (Dupont)—little or no localization.

(7) Pontacyl Fast Blue Conc. GR (Dupont)—little or no localization.

(8) Pontamine Fast Scarlet 4Ba (Dupont)—similar to Evans Blue and Lithium Carmine in localizing properties, although somewhat more toxic.

(9) Pontamine Sky Blue 5B—similar to Pontamine Sky Blue 6B.

A discussion of the chemistry of these dyes cannot be presented here. In a later communication, certain theoretical questions involved in the structure and preparation of localizing dyes will be considered.

In this connection, we wish to express grateful acknowledgment to Dr. W. C. Crossley of American Cyanamid, Dr. R. E. Rose of Dupont, and Mr. F. W. Green of National Aniline for their generous coöperation with us in preparing these various dyestuffs.

We wish also to express our gratitude to Dr. F. S. Cooper of the Haskins Laboratories for his continued and most helpful interest in this work.

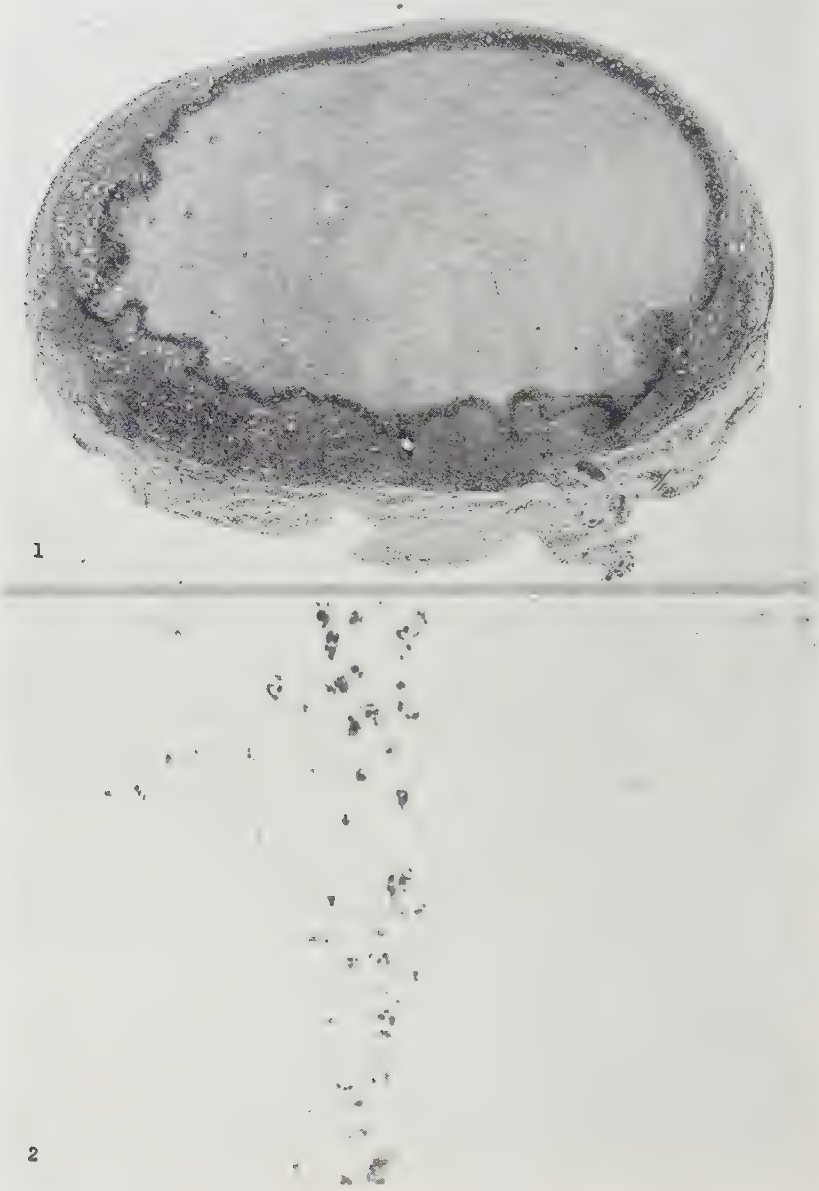

FIG. 1.

Photomicrographs of cross-section of mouse mammary carcinoma to show: (1) Dye localization at viable-necrotic interface. The viable periphery is separated from the necrotic center by a broad dark zone of localized dye. (2) Dye localization at viable-stroma interface. Dye droplets in phagocytes and fixed tissue cells at interface of growing tumor edge (right) and surrounding stroma (left).

Blue and Lithium Carmine. Nor were any differences noted in the localization of the dyes in spontaneous or transplanted tumors.

Histological Observations. The initial localization of either dye appeared as a diffuse faint staining of the stroma cells and interstices at the periphery of the tumor transplant. This zone was usually 1-2 mm in width. Wherever in the interior of the tumor an interface between viable and necrotic tissue occurred, a band of diffusely dyed cells, largely necrotic tumor cells and debris, was found (Fig. 1). This was especially prominent in the case of spontaneous tumors with numerous ramifying cores of necrotic tissue. Endothelial cells of capillaries at the periphery of the tumor were often seen diffusely dyed, especially if there was frank evidence of inflammation in the region.

Within a few hours after injection, and lasting for many days, macrophages and fixed tissue cells laden with dye granules were found at the periphery of the tumor (Fig. 2). These were present in large numbers after the first 24 hours. The diffuse staining of the capsular stroma gradually disappeared, usually within 48 hours. At the edge of the central necrotic core a few phagocytes containing dye were observed. They were not nearly as numerous, however, as those at the periphery. The necrotic core of the tumor often became slightly and diffusely stained, remaining so many days after injection. An occasional dye-filled phagocyte or a diffusely stained tumor cell could be found in the midst of the viable tumor cords. But the bulk of the malignant tissue remained free of dye throughout the procedure.

The dye substances were also rapidly taken up from the blood stream by the Kupffer cells of the liver, the reticulo-endothelial cells of the spleen and the renal tubule cells. Some localization occurred in the fixed tissue cells and macrophages of the dermis and to a lesser degree in the lungs and intestine. However, several days after injection, the reticulo-endothelial cells of the spleen and liver were found to be relatively clear of dye, although much was still present in phagocytes about the tumor. As long as dye remained in the segregating cells of the tumor mass, which was at least 3 weeks with the doses and tumors used, there was a slow excretion via the kidneys where the dye could be seen in the tubular epithelium, as well as in the urine. A precise quantitative estimate of the comparative concentration of dye in the various organs is not possible from histological preparations.[3, 4]

As far as one could tell a single massive dose resulted in about as much localization of dye in the tumor as the same dose given in small daily repeated fractions. Furthermore, the reticulo-endothelial system soon excreted the dye of a massive dose, but was continuously blocked when repeated small doses were given.

Collateral Observations. A number of experiments were performed in which 2 tumors were implanted bilaterally in the same animal, and one was irradiated with X-rays, doses varying from 20 to 1000 roentgens.[‡] The mice were then injected with dye at various time intervals following irradiation, and the X-rayed tumors compared with the normal. There was no evidence to indicate that external radiations strong enough to kill most of the tumor increased or decreased the localization of dyes in the tumor or other regions of the field. Nor did doses which caused slight cytological changes in the tumor cells appreciably alter the deposition of the dye.

Other experiments were performed in which diluted India ink was injected into tumor-bearing mice. There was no macroscopic or microscopic localization of ink either in the tumor or in the tumor area, although the ink was quickly segregated by the reticulo-endothelial system, the liver, spleen, lungs, and bone marrow taking up various amounts of the substance. This failure of colloidal carbon to localize in or around experimental tumors is almost the reverse of dye localization, and is confirmatory of the observations of Duran-Reynals[1] and Brunschwig, Schmitz and Clarke.[2] In an effort to seek an explanation for this behavior variance, India ink colloid solutions, as well as those of Evans Blue and Lithium Carmine, were subjected to centrifugation of 50,000 to 90,000 times gravity for 30 minutes. Such forces caused no observable sedimentation of the dye materials, but caused a considerable sedimentation of carbon particles from the ink solution. This may indicate a colloidal particle size difference in the two classes of materials, and suggests that their localization properties and passage through capillary membranes may be associated with relative colloid particle size.

Comment. It seems apparent, as pointed out by Brunschwig, Schmitz and Clarke[2] that the selective localization of dye in neoplastic foci of man, as in the case of animals, is dependent on some factor associated with a local increase in capillary permeability to the dye. According to the same authors, Evans Blue stains malignant neoplasms and their metastases much more heavily than benign neoplasms in rabbits and rats, and there seems to be no correlation between the histologic type of a neoplasm and its ability to localize the dye. The majority, although not all, of human patients with various types of carcinoma and sarcoma showed distinctive localization of intravenously injected dye.[2] However, since malignant cells *per se* do not take up injected colloidal materials, it would seem that the localization concept under examination would not be ideal for irradiation usage. On the other hand, there are several considerations which argue against such a conclusion. (a) The penetration paths through tissue of the various available energy particles are in most cases considerably greater than the thickness of a single cell. For electrons from radio-active isotopes the penetration may be as much as a centimeter or even more; for protons from the lithium-neutron reaction it is 50-60 micra; for alpha particles from the boron-neutron reaction it is somewhat less than 10 micra. Therefore, dye or other localizing substances containing materials which emit or can be caused to emit ionizing particles, when lying in juxtaposition with the outside of the growing tumor edge, or at any interface between necrotic and viable tissue, would tend to shower adjacent malignant cells with their ionizing radiations. (b) There is some evidence which indicates that therapeutic radiation effects on neoplasms are not due wholly to damage of the malignant cells *per se*. (c) Animal tumors, as well as most human cancers so far tested[2] not only accumulate a larger quantity of injected colloidal dye material than do normal tissues and vital organs, but in the course of metabolic excretion malignant neoplasms, experimental and spontaneous, retain such accumulations for a longer period than do the normal tissues of the body.

[‡] 190 kv, 30 m.a. Filters: 0.5 mm Cu, 1.6 mm Al. Target distance: 20 cm. 193 r per minute.

It seems, therefore, that if dye colloids, or other materials acting like them, are used as "carriers" for the selective deposition of either (a) ionizing short-lived radioactive isotopes of such elements as phosphorus, selenium, rubidium, etc., of (b) non-ionizing slow neutron-capturing materials, such as lithium or boron, a substantial internal radiation advantage which may prove of therapeutic value should be achievable.

Summary. (1) As earlier workers have shown, acid dyes do not localize in tumor cells but in the stroma surrounding the tumor. (2) Further localization takes place within the tumor at each interface of viable and necrotic tissue. (3) The initial localization consists of a diffuse staining of the peripheral stroma and the seminecrotic cells within the tumor between viable and necrotic zones. (4) This diffuse dye is progressively phagocytized, and after 24-48 hours, appears in numerous macrophages at the tumor edge. (5) The dye is so located that if it were replaced by a radioactive substance, theoretically effective radiation of the tumor edge should result. (6) After a single large dose of dye the reticulo-endothelial cells of liver and spleen are rapidly filled with dye droplets. These disappear or diminish substantially after several days. (7) In addition to tumor and reticulo-endothelial systems of liver and spleen, the kidneys take up considerable quantities of dye, as this is the chief channel of excretion. Accumulation in the kidney might prove to be a complicating factor in the use of radioactive materials, but in the case of induced activity, as with lithium by slow neutrons, appropriate shielding during irradiation should overcome this difficulty. (8) Small repeated doses of dye do not localize in greater quantities than does the same amount given at a single injection. Furthermore, the reticulo-endothelial system is blocked by small repeated doses. (9) X-ray treatment does not appreciably alter the amount of dye localizing in tumors, as determined by histological examination. (10) There is a marked difference in behavior, as regards tumor localization, between the colloidal dyes used and colloidal carbon. (11) Brief preliminary report is made on the localizing properties of some heretofore untested dyes.

13304

Immunological Reaction of the Hamster to Inoculations with the Virus of St. Louis Encephalitis.*

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The immunological response of mice to inoculation with St. Louis encephalitis virus has been rather extensively studied by Hodes and Webster.[1]

These authors[1] observed that mice inoculated subcutaneously with the virus failed to develop clinical evidences of encephalitis, but quickly developed immunity against subsequent intracerebral inoculation. This immunity, however, was not accompanied by the appearance of virus-neutralizing antibodies in the peripheral blood. Such antibodies were noted some 8 weeks after subcutaneous inoculation at which time, however, immunity to intracranial inoculation was lost.

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* Read before the meeting of the St. Louis Section of the Society for Experimental Biology and Medicine, May 14, 1941.

[1] Hodes, H. L., and Webster, L. T., *J. Exp. Med.*, 1938, **68**, 263.

We have shown[2] that the Syrian hamster *(Mesocricetus auratus)* is susceptible to inoculation with the virus of St. Louis encephalitis. This observation has been confirmed by Lennette.[3] Since the hamster is considerably larger than the mouse, it is possible to secure blood specimens repeatedly from the same animal and so follow the appearance of virus-neutralizing antibodies. The time of appearance of such antibodies is of clinical importance since it may indicate how rapidly virus neutralization tests may be expected to become positive during the course of human infection.

Virus neutralization tests were carried out according to the technic previously described by Broun and Ruskin,[4] which is similar to that of Webster and Fite.[5]

With each series of tests a non-immune serum was used as a negative control and a known immune serum as a positive control. The highest virus dilution in which two-thirds of the animals died in the non-immune serum control was considered as one lethal dose. This usually was in the 1/1,000,000 dilution. A serum which protects two-thirds of the animals against this quantity of virus, but not against greater quantities would be considered to have a titer of one minimal lethal dose protective unit. If a given serum protected two-thirds of the animals in all dilutions including the 1/5,000 virus dilution such a serum would have a titer of 200 minimal lethal dose protective units. No dilutions lower than 1/5,000 were used in the present study. Hence, 200 protective units is not necessarily the maximal titer of the sera studied. This degree of protection is, however, comparable with the more potent human convalescent sera which we have collected from patients who have recovered from St. Louis encephalitis.

Prior to inoculation, the serum of all hamsters so far studied contains no protective antibodies against St. Louis encephalitis virus.

Subcutaneous inoculation of 2 cc of 1/10 dilution of encephalitic mouse brain in no instance has been followed by the appearance of evidence of encephalitis. Several animals in this series died as a result of the cardiac puncture used to secure the blood specimens.

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[2] Broun, G. O., Muether, R. O., Mezera, R. A., and LeGier, M., Proc. Soc. Exp. Biol. and Med., 1941, **46**, 601.

[3] Lennette, E. H., Proc. Soc. Exp. Biol. and Med., 1941, **47**, 178.

[4] Broun, G. O., Ruskin, J., *J. Mo. State Med. Assn.*, 1936, **33**, 19.

[5] Webster, L. T., and Fite, G. L., *Science*, 1933, **78**, 463.

Animals inoculated in this manner show prompt and intense development of humoral antibodies. A serum antibody titer capable of neutralizing 200 lethal doses of virus developed in 4 out of 7 animals as early as 48 hours after inoculation and in 3 other animals this level was reached by the third day. None of the animals which were inoculated subcutaneously with virus failed to show at least some protective power in all serum specimens collected after the first 48 hours. Once the high titer of 200 neutralizing doses is reached, it persists in most animals at this level for periods as long as 24 weeks.

All animals reinoculated intracerebrally as long as 24 weeks after subcutaneous inoculation have survived as much as 10,000 lethal doses. Humoral antibodies in a titer of 200 lethal dose protective units were present at the time of this reinoculation in each animal.

Twelve animals were studied after intracerebral inoculation of .06 cc of 1/10 to a 1/1,000,000 dilution of encephalitic mouse brain. All except 2 resistant animals succumbed to the infection within 4 or 5 days. Three of these animals, bled when moribund, show no neutralizing antibodies in their serum. Some of the other animals as early as the fourth day after inoculation developed antibodies of such titer that the serum will protect against from 10 to 200 lethal doses. Even the latter titer does not prevent the fatal termination of the infection.

Two resistant animals were observed which did not succumb to the intracerebral inoculation. One of these animals developed antibodies capable of neutralizing only 10 lethal doses on the eighth day after inoculation. Later this animal showed protection against 100 lethal doses on the sixteenth day after inoculation and against 200 lethal doses on the twenty-first and thirtieth days after inoculation. The animal then died as a result of the cardiac puncture used in securing the blood specimen. In this case the titer of protective antibodies appeared to develop very gradually. A second resistant animal whose serum was not examined until 12 days after inoculation showed at that time 200 protective units. He has maintained this level, with the exception of a temporary drop during the third week for over 12 weeks.

When inoculated intranasally with .06 cc of 1/10 to a 1/1,000 dilution of encephalitic mouse brain 7 out of a group of 9 hamsters succumbed to the infection between the fourth and seventh days. Three animals showed no protective antibodies when bled immediately prior to death as late as the sixth day after inoculation. Other animals from 3 to 7 days after inoculation developed antibodies capable of neutralizing from 10 to 200 lethal doses of virus and still succumbed to the infection. Two resistant animals survived even though a serum titer of only one and 10 protective units was developed within the first week after inoculation. By the fourth week these animals had developed a titer of 200 protective units and it was maintained for at least 9 weeks at this level with only occasional fluctuations.

We have so far studied only 2 animals after feeding the virus orally. Blood withdrawn from one of these 8 days after the feedings began showed a serum titer of only one protective unit. The animal died as a result of the cardiac puncture. The brain contained no demonstrable virus. The second animal showed a serum titer of 10 protective units on the twelfth and again on the nineteenth day after the feedings began. This animal still survives in good health. It would, therefore, appear that oral feeding can excite some immunological response in these animals although we have not yet seen a high titer of neutralizing antibodies so produced.

By all routes of inoculation all animals which survived as long as 7 days show at least some neutralizing power in their blood serum. In no instance has protective power been completely lost in animals surviving the first week during periods of observation as long as 14 weeks. One animal inoculated intranasally showed 10 protective units 3 days after inoculation but on the fifth day the serum failed to show protective power. The animal died on that day.

Summary. Humoral antibodies against the virus of St. Louis encephalitis can be demonstrated in the sera of hamsters inoculated by subcutaneous, intracerebral, intranasal and oral routes provided the animals survive the infection 7 days or more.

After subcutaneous inoculation, antibodies may appear in the serum within 48 hours after inoculation. They persist at high titer for many weeks. When inoculated intranasally or intracerebrally, some animals fail to develop antibodies prior to death and others which develop a high protective titer still succumb to the infection. Animals dying as long as 6 days after intracerebral or intranasal inoculation may fail to show neutralizing antibodies. No animal inoculated subcutaneously has failed to show humoral antibodies after the second day. The presence of humoral antibodies does not insure immunity against the disease.

13305

Presence of Androgens in the Placenta.

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From the Department of Zoology, Duke University.

Although the presence of androgenic substances in the placenta is mentioned by several authors, these fail to make any specific citation to the original work, and an examination of the more common indices is no more encouraging.

Siebka[1] examined 2 placentae following operative abortion and failed to find any androgens in the 4-5-month-old organs. If there is any positive evidence of the occurrence of androgens in the placenta, it has not found its way into the current literature.

The purpose of this study was to determine whether or not the presence of androgens in the placenta could be demonstrated.

Three male and 3 female full term placentae were secured, washed as free of blood as possible and extracted according to the Gallagher and Koch method. The extracts were assayed both on capons and by the colorimetric methods.

The colorimetric assay* indicated the presence of one international unit in 60.7 g of male placenta, and one I.U. in 69.4 g of female placenta. The capon test showed for the male placentae one I. U.

TABLE I.

Placenta No.	Wt, g	Capon No.	Total comb growth		I.U.	Grams tissue per I.U.	Colorimetric grams tissue per I.U.
1 (male)	500	1	5	7.3	6.8*	226.9	60.7
2 "	470	2	2.3				
3 "	410						
Total wt	1380						
4 (female)	470	1	2.6	4.4	3.66*	371.5	69.4
5 "	425	2	1.8				
6 "	465						
Total wt	1360						

*Corrected according to McCullagh and Cuyler, *J. Pharm. and Exp. Ther.*, 1939, **66**, No. 4.

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[1] Siebka, H., *Arch. f. Gynäkol.*, 1931, **146**, 415.

* For which we thank W. K. Cuyler, of the Duke Hospital.

per 226.9 g of tissue; the female one I. U. per 371.5 g. Only two birds were used for each test and the difference in response was in one case about 100%, in the other about 50%. Lack of material prohibited the use of more birds.

While the experiments give positive evidence of androgens in the placenta, they may not be taken as quantitative. The wide variation between the colorimetric test and the capon assay is confusing, but may be related to the relative amounts of androgens or 17 ketosteroids. No effort was made to determine which of the 17 ketosteroids were present.

It is rather interesting, although it may not be significant, that the male placenta contains considerably more hormone, by both tests, than the female.

13306

Implantation Following Mating in Hypophysectomized Rats Injected with Lactogenic Hormone.*†

EUGENE CUTULY.

From the Department of Anatomy, Wayne University College of Medicine, Detroit.

In a recent article[1] the writer reported that lactogenic hormone, hypophyseal synergist, and whole anterior pituitary extract maintained pregnancy in rats hypophysectomized after implantation of fertilized ova had occurred. The data which follow may show that nidation and subsequent development of fertilized ova will occur in hypophysectomized rats under the influence of lactogenic hormone.

Fourteen experimental animals were used. Time of mating was determined by the presence of a copulation plug in the vagina or of sperm observed in the vaginal smears. Vaginal examinations were made at 4:00 p.m. and 10:00 a.m., so the exact time of coitus, which always occurred in the intervening 18-hour period, was not

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* This study was aided in part by Works Project Administration No. 665-51-3-225.

† Most of the lactogenic hormone used in this work was supplied by Dr. Erwin Schwenk, Schering Corporation, Bloomfield, New Jersey. Some of the lactogenic hormone was supplied by Dr. J. P. Schooley, Difco Laboratories, Inc., Detroit, Michigan.

[1] Cutuly, E., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 126.

known. If vaginal examination showed that coitus had occurred during this interval, the female was considered to be 1 day pregnant, even though fertilization could not have occurred more than 18 hours previously. In many instances, in fact, it is probable that signs of coitus were discovered very shortly after mating, as frequently large numbers of motile sperm were found in the vaginal smears. Since hypophysectomies were never performed after 4:00 p.m., it follows that the interval between mating and hypophysectomy (Table I, column 2) was invariably shorter than indicated. The method described for determining inception of pregnancy has been employed by the writer for a number of years on a large number of normal rats. Its accuracy is indicated by the fact that delivery in such animals has almost without exception occurred on the 22nd day post coitum; *i. e.*, 21 days after discovery of sperm in the vagina.

In 4 animals (Nos. 1-4) injected with lactogenic hormone[2] (1-3 mg daily) implantation did not occur.

In 8 animals (Nos. 6-13) in which implantation was successful,

TABLE I.
Influence of Lactogenic Hormone on Rats Hypophysectomized at Pre-implantation Stages of Pregnancy.

		Treatment						Autopsy data	
Rat No.	Hypophysectomy after mating (days)	Substance	Daily dose in 1 cc (mg)	No. of days continued	First blood in vagina, days after mating	Pregnancy maintained days after mating	No. in litter	Ovaries (mg)	Adrenals (mg)
1	4	SL*	1	19	0	0	0	27	27
2	3	SL	3	16	0	0	0	44	41
3	1	SL	3	16	0	0	0	25	29
4	2	SL	3	19	0	0	0	30	30
6	5	DL35†	3	18	10	9	0	33	22
7	4	SL	3	18	10	9	0	21	31
8	3	DL35	1	17	7	6	0	32	27
9	4	SL	3	15	10	9	0	26	31
10	3	SL	1	11	10	9	0	29	33
11	3	SL	3	10	11	10	0	31	38
12	4	SL	3	14	12	17	8	31	30
13	1	SL	3	19	—	‡	10	50	40
15	3	DL786B§	3	20	13	23	7‖	42	33
16	3	SL	3	23	12	25¶	8	49	36
Avg of 4 normal pregnant controls					13	22	7	75	65
Avg of 7 normal non-pregnant controls								69	61

*SL = Schering Prolactin, assaying 12½ I.U. per mg.
†DL35 = Difco Prolactin, assaying 10 I.U. per mg.
‡At autopsy small well formed fetuses were found. Possibly implantation was delayed.
§DL786B = Difco Prolactin, assaying 10 I.U. per mg.
‖Young dead at birth.
¶Rat died without delivering.

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[2] Evans, H. M., Simpson, M. E., and Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 586.

pregnancy was interrupted 6 to 17 days after impregnation. Hypophysectomy was performed 1 to 5 days after mating, and the animals were treated with lactogenic hormone (1-3 mg daily). In all but one (No. 13) resorption of embryos or fetuses was in progress at the time of autopsy. Unfailing evidence that resorption was under way was provided by the presence of blood in the vagina prior to the expected time of the "placental sign," and by pronounced and progressive loss in body weight. In the table the beginning of fetal resorption is given as one day prior to the appearance of blood. This arbitrary figure might not be correct in all instances, but it serves as a relative measure of time during which pregnancy was maintained in the group of animals. At autopsy, swellings of various sizes were seen along the uteri. These sites were found to contain hematomata surrounding embryos or fetuses and sometimes placentae which had undergone different degrees of degeneration. Rat No. 13 deserves special mention. It had lost weight after operation, although it showed sporadic small gains. Except for a faint brownish coloration in the vaginal mucus 17 days after mating, no blood was observed when vaginal smears were made. On post coital day 21 the animal was killed. Five swellings were found in each uterine horn, which upon dissection revealed well formed fetuses of different sizes. These were judged to be comparable to normal fetuses of 9 to 12 days of age. Possibly implantation had been delayed.

In 2 animals (Nos. 15 and 16), hypophysectomized 3 days after mating, pregnancy was continued to term (No. 15) or beyond term (No. 16) under the influence of lactogenic hormone (3 mg daily). Rat No. 15 delivered 7 dead fetuses on day 23 of pregnancy, while rat No. 16 died 25 days after mating without delivering. At autopsy 8 large dead fetuses were found in the uterine horns. The mammary glands in both cases were highly developed and the ovaries contained large healthy corpora lutea.

Ovarian weights were low compared with normal values. This would seem to indicate lack of stimulation except possibly that of previously formed corpora lutea. On the basis of weight, it seems possible that the adrenal glands of some of the treated animals were slightly stimulated. This matter, however, must be investigated more thoroughly before the heavier weights of some of the glands can be considered significant.

The results presented, together with those recently published,[1] leave no doubt that lactogenic hormone is capable of stimulating function of the corpus luteum. Evans and coworkers[2, 3] have

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[3] Evans, H. M., Simpson, M. E., Lyons, W. R., and Turpeinen, K., *Endocrinology*, 1941, **28**, 933.

illustrated this action of lactogenic hormone by production of deciduomata in hypophysectomized rats, and Astwood[4] has employed mucification of the vagina in immature rats as a means of demonstrating the same effect. When gonadotropic substances[1] are employed in attempts to stimulate function of the corpus luteum, the question of dosage must be regarded as highly important. Dosage of a gonadotropin which can cause follicular as well as luteal development is difficult to regulate. If the dose is low only follicles form; if the dose is high both follicles and corpora lutea develop. Demonstration of luteal function in such instances is difficult since the output of estrogen would tend to override effects of luteal hormone. Until such problems are more thoroughly investigated, therefore, it does not seem wise to reject the view that some gonadotropins which cause formation of corpora lutea may also cause the corpora to function.

Summary. Fourteen rats, hypophysectomized 1 to 5 days after mating, were injected with lactogenic hormone (1 or 3 mg daily). In 4 animals implantation failed to occur. Of the remaining 10 animals 2 carried to term or beyond, while pregnancy was interrupted after 6-17 days in 8 rats. It would seem that lactogenic hormone was capable of stimulating corpus luteum function sufficiently to induce implantation and to maintain pregnancy for periods ranging from 6 days to term or beyond.

13307

Chemotherapy of Lymphogranuloma Venereum with Sulfonamide Drugs.

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It was recognized early that in lymphogranuloma venereum, unlike most other virus diseases, some success attended treatment of infected mice or guinea pigs with drugs of the sulfonamide group. The earlier work has been summarized by Findlay.[1] In a previous communication from this laboratory[2] it was indicated that, in preliminary experiments, sulfapyridine and sulfathiazole given orally in the form of the free acid twice daily after infection were equally effective in preventing death of mice infected intracerebrally. In these experiments mouse brain was used as inoculum, and even with a 1 in 10 suspension only 63% of the control mice died. In subsequent papers Findlay[1, 3] repeated his earlier results with sulfanilamide, showing a reduction of mortality from 84% in the controls to 49% in those fed sulfanilamide in gum acacia daily.[3] He further attempted to assess the relative value of sulfanilamide, sulfapyridine, sulfathiazole, sulfamethylthiazole and disodium 4:4 bis-o-carboxybenzoylaminodiphenylsulfone.[1] The mice received 10 mg per 20 g of body weight in 2 equal daily doses orally by stomach tube for the first 4 compounds and subcutaneously for the last. One hundred mice were treated with each compound, together with 100 controls, and the ratio of treated survivors to control survivors was used as a method of comparing one experiment with another. On the basis of his results, Findlay maintained that the order of activity ran: sulfamethylthiazole > sulfapyridine > sulfathiazole > sulfanilamide > lutazol. Smaller experiments with compounds inoculated intramuscularly in olive oil indicated slight activity for ammonium 4-nitrobenzene-sulfonate and 4:4-dinitrodiphenylsulfone.

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[4] Astwood, E. B., *Endocrinology*, 1941, **28**, 309.

[1] Findlay, G. M., *Lancet*, 1940, **2**, 682.

[2] McKee, C. M., Rake, G., Greep, R. O., and van Dyke, H. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 417.

All of the previous work had been carried out with virus material of low titer derived from infected mouse or monkey brain. The higher titer which could be achieved with infected yolk-sac[4] gave rise to the hope that with such material more conclusive evidence of the relative efficacy of the different sulfonamide drugs could be obtained than was possible with material which killed at best only about 80% of the control mice when very high concentrations of tissue were used.

In the early experiments reported here the inoculum was prepared by grinding heavily infected yolk sacs in a mortar with quartz. This material gave an L_D50 of 1/56 for 10 to 12 g mice when 0.03 cc were given intracerebrally, and titered 10^{-8} to 10^{-9} by yolk-sac inoculation of eggs. In later experiments the heavily infected yolk sacs were shaken, without beads, in a shaking machine in the presence of sufficient broth to make a 10% suspension. Such treatment liberated all virus by rupture of the yolk cells but left the greater part of the chick tissue in a tight ball at the bottom of the suspension. With this material the L_D50 for mice inoculated with 0.03 cc intracere-

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[3] Findlay, G. M., *Brit. J. Exp. Path.*, 1940, **21**, 356.

[4] Rake, G., McKee, C. M., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 332.

TABLE I.

Results of Chemotherapy with Sulfonamide Drugs in Lymphogranuloma Venereum of Mice.

Infective dose		Sulfanilamide Deaths to		Sulfathiazole Deaths to		Sulfapyridine Deaths to		Sulfaguanidine Deaths to		Sulfadiazine Deaths to		Normal diet Deaths to	
		10 d	4 wk	10 d	4 wk	10 d	4 wk	10 d	4 wk	10 d	4 wk	10 d	4 wk
Exp. 1	1/10											5/5	5/5
Ground	1/50	0/10	*3/10	0/10	0/10	7/10	7/10	2/10	3/10			9/10	9/10
Virus	1/100											4/9	6/9
	1/500					(0.5% diet)						0/10	0/10
	1/1000											0/10	†4/10
Exp. 2	1/10	10/10	10/10	7/10	7/10	10/10	10/10	2/10	2/10			10/10	10/10
Ground	1/50	2/10	2/10	1/10	1/10	0/10	0/10	1/10	1/10			6/10	6/10
Virus	1/100											2/10	3/10
Exp. 3	1/10	16/20	17/20	15/20	15/20	16/20	16/20	10/20	11/20			10/10	10/10
Ground	1/50											5/10	5/10
Virus	1/100											2/10	3/10
Exp. 4	1/50	9/20	10/20	8/20	8/20	10/20	10/20	7/20	7/20			10/10	10/10
Shaken	1/500											14/20	14/20
Virus	1/1000											7/20	7/20
	1/5000											1/20	3/20
Exp. 5	1/50	5/20	5/20	7/20	8/20	9/20	10/20	5/19	5/19	20/20	20/20	8/10	9/10
Shaken	1/500											4/19	4/19
Virus	1/1000											2/19	2/19
	1/5000											1/20	1/20
Exp. 6	1/50			6/20	7/20					1/20	3/20	8/8	8/8
Shaken	1/500											3/10	3/10
Virus	1/1000									(0.1% diet)		0/10	1/10
Exp. 7 Shaken Virus	1/50	23/40	25/40	17/40	17/40	35/40	35/40	19/39	20/39	20/40 (0.1% diet)	22/40	10/10	10/10

Except where indicated, the drug was incorporated as 1% of the diet.

*3/10 indicates that of 10 mice in this group 3 died.

†Probably died of intercurrent infection.

brally was 1/416 and the titer of yolk-sac inoculation was increased approximately tenfold.

Treatment with the sulfonamide drugs intimately mixed as 1% of No. 1 Sherman dry diet[2] was commenced 24 to 48 hours before the infecting dose was given in order that the mice might become accustomed to the food. Treatment was continued for 10 days after infection. The results are shown in Table I.

With inoculum prepared by grinding, a dose of 1:10 killed all the control mice within 10 days (*i. e.*, from acute infection; later deaths up to 4 weeks were usually due to hydrocephalus). Sulfaguanidine gave the best results followed by sulfathiazole, sulfanilamide and sulfapyridine. The 1:50 dose, which killed 75% of the controls, was apparently too low to show conclusive differences between the different drugs.

With inoculum prepared by shaking, sulfadiazine* at 0.1% was the most effective drug, followed by sulfathiazole, sulfaguanidine, sulfanilamide and sulfapyridine. With sulfadiazine at 1% all the mice died (see experiment 5) with an average survival time of 73 hours. That their deaths were due to combined toxicity of drug and infection is suggested by the fact that in our hands, contrary to previous reports,[5] only 4 out of 9 mice on 1% sulfadiazine diet for 30 days died, and the earliest death in this group was on the 10th day. Twelve mice receiving 1% sulfadiazine diet showed a blood level of total sulfadiazine from 21.6 to 43.1 mg % (free 17.5 to 31.0 mg %) which is 7 to 8 times the figures obtained on an equal number of mice fed 1% sulfathiazole. With 0.1% sulfadiazine in the diet the blood levels were: total 5.6 to 6.4 mg %, free 5.3 to 6.2 mg %, and this concentration in the diet was therefore chosen for future experiments.

It is thus clear that drugs of the sulfonamide group, and particularly sulfadiazine, sulfathiazole and sulfaguanidine are capable of preventing death from acute infection with lymphogranuloma venereum when given prophylactically. Preliminary experiments on the therapeutic administration of the drugs in the diet (in which the inoculated mice, directly after inoculation, are placed in cages containing the appropriate drug-diet mixture) show that the drugs are just as effective in preventing death under these conditions. Thus in experiments with shaken virus at 1:50, 14 out of 15 control mice on normal diet died within 10 days compared to 5 out of 25 mice receiving 1% sulfathiazole diet and 4 out of 24 receiving 0.1% sulfadiazine diet.

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* We wish to acknowledge the kindness of Mr. F. M. Fargo, Jr., and Dr. M. L. Crossley of the Calco Division of the American Cyanamid Company for supplying us with the sulfadiazine used in these experiments.

[5] Feinstone, H. W., Williams, R. D., Wolff, R. T., Huntington, E., and Crossley, M. L., *Bull. Johns Hop. Hosp.*, 1940, **67**, 427.

While the action of the sulfonamide drugs in preventing death from acute infection is thus incontravertible, it was noticed that in practically no case did any mouse, treated or untreated, remain free from symptoms of disease. These symptoms are completely lost within 2 weeks except in those mice which have obvious chronic hydrocephalus with domed cranium and progressive loss of weight. In view of the fact that most humans infected with lymphogranuloma venereum remain Frei positive and show high complement fixing antibodies despite sulfonamide therapy,[6] and that, in our opinion, such persistence of skin sensitivity and serum antibodies probably indicates persistence of infection in a latent form, it seemed important to study the possibility of a carrier state in recovered mice, whether drug-treated or not. Proofs of the persistence of the virus in sick mice for periods up to 43 days after infection have already appeared in the literature.[7–10] In some cases these were control mice and in others mice which had received sulfonamide drugs. In our own experience, mice have been allowed to live for 3½ months after infection. They developed normally and the females produced and reared healthy families. When sacrificed, a certain number of the animals showed varying, but not crippling, degrees of hydrocephalus which was slightly more frequent in the controls (4 out of 6 animals) than in those which had received drugs (6 out of 12 for sulfaguanidine and 2 out of 8 for sulfathiazole). Virus was isolated from the brains of mice in all 3 groups and demonstrated by yolk-sac inoculation. Small amounts of virus were also demonstrated in the pooled spleens from the sulfaguanidine-treated group in these early experiments. A strain of virus recovered from the brains of the sulfaguanidine-treated mice was studied further. It showed a decreased virulence for eggs by yolk-sac inoculation (10-fold) and for mice by intracerebral inoculation (5-fold), and this decreased virulence persisted for 3 egg passages, which was as long as it was studied. Schlossberger and Bär[9] also comment on the decreased virulence of the strains recovered from convalescent or sick mice.

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[6] Harrop, G. A., Rake, G., and Shaffer, M. F., *Trans. Assn. Am. Phys.*, 1941, in press.

[7] MacCallum, F. O., and Findlay, G. M., *Lancet*, 1938, **2**, 136.

[8] Levaditi, C., *Compt. rend. Soc. biol.*, 1938, **129**, 490.

[9] Schlossberger, H., and Bär, F., *Zbl. Bakt.*, 1939, **144**, 228.

[10] Bär, F., *Z. f. Immunitätforsch. u. exp. Therap.*, 1940, **97**, 344.

It would appear, therefore, that while the sulfonamide drugs are able to prevent deaths from acute infection with lymphogranuloma venereum, their action is not always curative. Recovered mice may live healthy lives and raise families over periods of 3½ months, but at the end of this time show signs of chronic hydrocephalus and yield active, although slightly less virulent, strains of virus from the brain and other tissues. The importance of this in human chemotherapy cannot be over-emphasized. The idea that patients, receiving such therapy and becoming clinically "cured", are cured despite persistence of positive Frei and complement fixation tests requires reconsideration. Studies are now under way on the relative sterilizing powers of the different drugs, on the duration of the carrier state, and on the possible transmission of the infection from infected mother to the foetus *in utero*.

13308

Sulfonamide Inhibition of Bacterial Luminescence.*

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Luminescence intensity is inhibited at once on addition of sulfanilamide either to broth cultures, or washed cell suspensions of luminous bacteria in phosphate buffered NaCl solutions containing glucose as oxidizable substrate. At the respective optimum temperatures of marine species, *Achromobacter Fischeri* (25 to 28°C), *Photobacterium phosphoreum* (15 to 17°C), and a fresh-water species, *Vibrio phosphorescens* (28 to 30°C), a concentration of 100 mg % sulfanilamide greatly reduces the intensity of luminescence, with little or no effect on the rate of respiration (Fig. 1). Except at high concentrations of the drug the inhibition of luminescence is largely or completely reversible (Table I) by centrifuging and resuspending the cells in a drug-free solution. In all these respects the effects of sulfanilamide resemble those of typical narcotics, such as the urethanes[1, 2] and barbital.[3] By analogy, it would be predicted that sulfanilamide will inhibit the luminescent

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* Aided in part by a grant from the Penrose Fund of the American Philosophical Society.

[1] Taylor, G. W., *J. Cell. Comp. Physiol.*, 1934, **1**, 297.

[2] van Schouwenburg, K. L., *On Respiration and Light Emission in Luminous Bacteria*, Thesis, Delft, 1938.

[3] Johnson, F. H., and Chambers, E. L., *J. Cell. Comp. Physiol.*, 1939, **13**, 263.

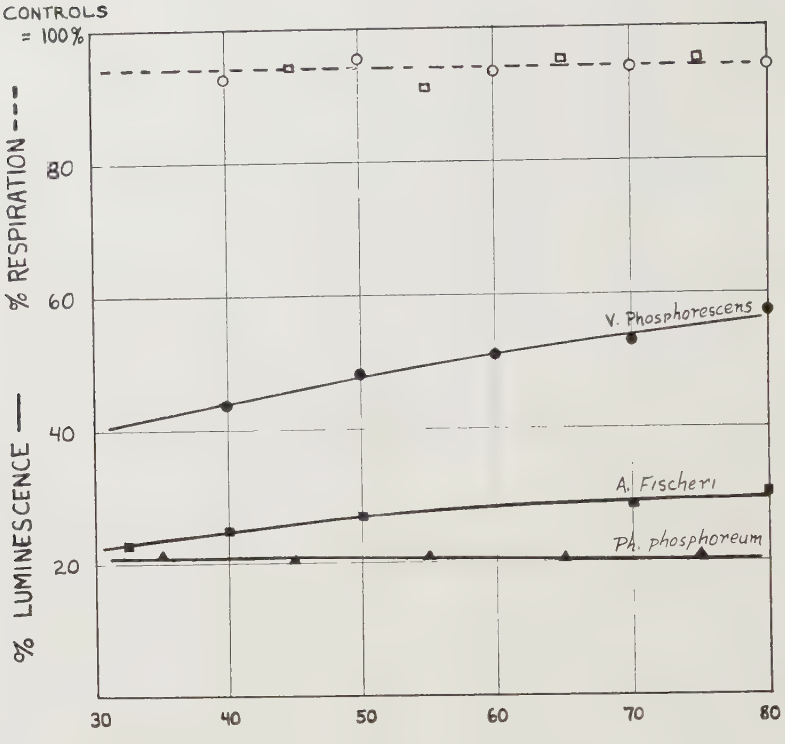

FIG. 1.

The effect of 100 mg % sulfanilamide on the luminescence intensity (solid points and lines) and rate of oxygen consumption (hollow points and broken lines) of three species of luminous bacteria at their respective optimum temperatures: *Photobacterium phosphoreum,* 15.5°C (triangles); *Achromobacter Fischeri,* 28°C (squares); and *Vibrio phosphorescens,* 28°C (circles). All suspensions were washed twice with NaCl solution of optimum tonicity (3% NaCl for the first two species, and 0.9% NaCl for the third) and resuspended in phosphate buffered NaCl solution, at a pH of 7.30, containing 0.01 M. glucose. The results are expressed as per cent with respect to a control without sulfanilamide, for both oxygen consumption (measured by Warburg respirometers) and for luminescence intensity (measured by a modified Leeds and Northrup MacBeth Illuminator).

oxidation of purified *Cypridina* luciferin and luciferase *in vitro.*[4]

Growth of luminous bacteria in nutrient broth is prevented by high concentrations of sulfanilamide (500 mg %), while lower concentrations retard growth and sometimes allow the development of cultures which do not luminesce. In sub-cultures of the latter, luminescence reappears, showing that no permanent change in the characteristics of the organism has taken place.

Concentrations of sulfanilamide which reduce luminescence intensity to as little as 10% cause neither an appreciable bactericidal

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[4] Harvey, E. N., *Erg. d. Enz. Forsch.,* 1936, **4**, 365.

TABLE I.

Inhibition of Luminescence Intensity on Adding Sulfanilamide to Broth Cultures of *Photobacterium phosphoreum* at 25°C, and the Reversal of Inhibition by Centrifuging, Washing Once with NaCl, and Resuspending the Cells in a Medium Without Sulfanilamide.

		At start		After washing out the sulfanilamide	
Vessel	Conc. sulfanilamide in mg%	Luminescence intensity (arbitrary units)	% inhibition	Luminescence intensity	% inhibition
1	0 (control)	26.0	....	18.8	....
2	50	9.5	63.4	15.3	19.7
3	0	26.0	....	17.5	....
4	500	1.3	95.0	3.9	77.7

TABLE II.

Effect of 250 mg% Sulfanilamide on the Luminescence Intensity and Viability of Cells in a 24-hour Broth Culture at 15°C of *Ph. phosphoreum*. Temperature of Cultures During Experiment Was 23.5°C.

	Control			Plus sulfanilamide		
		Plate count 1:400,000 duplicate plates			Plate count 1:400,000 duplicate plates	
Minutes after adding sulfanilamide to culture	Luminescence intensity (arbitrary units)	Total colonies	Luminous colonies	Luminescence Intensity (same units)	Total colonies	Luminous colonies
5-10	11.7	113	90	1.6	110	92
		121	90		92	82
20-26	11.5	121	90	1.2	115	75
		108	86		105	62
35-45	9.1	123	79	<1.0	112	88
		105	89		120	94
50-60	8.2	121	89	<1.0	105	60
		119	98		84	77
70-75	7.7	115	86	<1.0	113	89
		143	115		110	96

effect, nor a reduction in number of luminous colonies in the plate count (Table II).

Para-aminobenzoic acid[5] at high concentrations (50 mg %) inhibits growth and luminescence, but exerts anti-sulfanilamide effects on broth cultures at much lower concentrations. Para-aminobenzoic acid does not, however, have any appreciable effect on the sulfanilamide inhibition of luminescence in mature cultures or washed cell suspensions, over a range of 0.001 to less than 0.0000001 M. It thus appears that PAB acts primarily through an effect on growth, and cannot reverse the narcotising action of sulfanilamide on the luminescence of normal cultures.

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[5] Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

13309

An Improved Closure for the Doster-Virtue Intestinal Loop.*

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Specially treated bone was used to close the intestinal loop previously described.[1] Experience has shown that it is difficult to obtain uniform preparations of bone which will not eventually disintegrate after an exposure of some months to the action of intestinal juices. This difficulty has been overcome by substituting stainless steel wire mesh for bone. A ¾ inch width of 30 mesh stainless steel wire mesh† is wound around the rubber tubing until the desired thickness of "bone" is obtained. The rough ends are closed by vulcanizing rubber onto them, which thus fastens the wire to the tubing. A porous matrix through which the serosa can grow is thus established. Loops closed in this manner are intact after 6 months of use, and show no sign of leakage or disintegration. Vitalium wire mesh was also satisfactory but costs several times as much as stainless steel.

One other troublesome factor, that of the inserted end of the rubber tubing puncturing the walls of the loop, has been eliminated. A roll of rubber was vulcanized around the end of the tubing which was inserted into the loop at the time of operation, thus eliminating sharp edges of hard rubber. No punctures have occurred since this practice was begun.

13310

Sensitization of Blood Vessels to Acetylcholine by Sympathetic Denervation.

Hudson Hoagland. (Introduced by W. B. Cannon.)
From the Department of Physiology, Harvard Medical School.

Degeneration of the nerve supply to skeletal muscle and to certain smooth muscles contracted by sympathetic impulses is known to sensitize those effectors to chemical agents. This subject has been reviewed by Cannon and Rosenblueth[1] and by Rosenblueth.[2] Cannon and Haimovici[3] have demonstrated sensitization to chemical agents of motoneurons of the spinal cord after chronic hemisection, and Burrett[4] has found that the chronically denervated heart is sensitized to adrenaline.

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* The authors wish to thank Dr. R. W. Whitehead for use of the animal quarters at the Medical School of the University of Colorado.

[1] Doster-Virtue, M. E., and Virtue, R. W., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 813.

† W. S. Tyler & Co. of Cleveland, Ohio, furnished the wire mesh.

It was desired to see whether the contractile elements in blood vessels that are subject to cholinergic nerves could be sensitized to acetylcholine by denervation as blood vessels subject to adrenergic nerves are thus sensitized to adrenaline (Meltzer and Meltzer[5]). Experiments were accordingly carried out on the chronically sympathectomized blood vessels of the posterior limbs of cats, in which there is evidence for the presence of cholinergic vasodilator control (Rosenblueth and Cannon[6]).

Method. The abdominal sympathetic chain was removed unilaterally with aseptic precautions in 10 cats which survived the operation without complications. In each animal approximately 2 cm of the sympathetic chain, embracing usually 3 or 4 ganglia, were removed from deep in the pelvis. According to Langley[7, 8] sympathetic vasomotor fibers to the lower limb of the cat are supplied by the 6th and 7th lumbar ganglia (*i. e.,* 4th and 5th according to present nomenclature) and by the 1st and 2nd sacral ganglia. For degeneration of the nerve supply 20 to 44 days were allowed before testing for possible sensitization to acetylcholine. The cat was then anesthetized with dial, a tracheal cannula was inserted, the right or left external jugular vein was exposed for injection and the carotid artery was cannulated for blood-pressure recording. Both posterior limbs were inserted in glass plethysmographs for simultaneously recording leg volumes on the two sides, a rubber cuff making junction between leg and tube. The unshaven legs were sealed in the plethysmographs by vaseline, the remaining air displaced by water at a temperature of approximately 37°C. It was thus possible to obtain kymograms of blood pressure and limb-volume measurements as indices of the effects of intravenous injections of acetylcholine. Eserine was not used in any of the experiments. The response of the chronically denervated limb to injections was compared to that on the normal side, after which, in some cats, the remaining abdominal sympathetic chain was acutely removed and the experiments repeated. The acute sympathectomy had no systematic effect on the response of the chronically denervated limb as compared to the previous response. There was, moreover, no systematic difference in limb-volume response to acetylcholine measured before and after the acute sympathectomy.

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[1] Cannon, W. B., and Rosenblueth, A., *Autonomic Neuro-effector Systems,* New York, 1937.

[2] Rosenblueth, A., *Ann. Rev. Physiol.,* 1940, **2**, 263.

[3] Cannon, W. B., and Haimovici, H., *Am. J. Physiol.,* 1939, **126**, 731.

[4] Burrett, J. B., *Ibid.,* 1940, **131**, 409.

[5] Meltzer, S. J., and Meltzer, C., *Ibid.,* 1903, **9**, 252.

[6] Rosenblueth, A., and Cannon, W. B., *Ibid.,* 1935, **112**, 33.

[7] Langley, J. N., *J. Physiol.,* 1891, **12**, 347.

[8] Langley, J. N., *Ibid.,* 1891, **12**, 375.

After each experiment the plethysmographs were calibrated by the injection from a hypodermic syringe of measured volumes of water into the rubber tubes connecting the limb vessels with float microrespirometers which were used, with suitable levers, for the recording. Each plethysmograph gave displacements on the drum of approximately 16 mm per 0.5 cc and they seldom deviated from each other by more than 1 mm per 0.5 cc. For experients in which deviations of one or more millimeters per 0.5 cc occurred between the two plethysmographs, correction factors were applied to make the readings comparable.

Results. When all 4 of the abdominal ganglia in question (from the 6th lumbar caudad) are removed unilaterally, and when care is taken to avoid injury to the contralateral chain, there is clear evidence of sensitization to acetylcholine on the chronically denervated side. The importance of these conditions was not realized until negative results were obtained with a number of cats in which fewer than the 4 ganglia had been removed and in which possible damage to the contralateral chain during the operation was not ruled out.

Immediately after injection of acetylcholine the limb volume at first usually falls slightly and then increases markedly. The initial fall is variable and may be absent. It is possible that skin volume and muscle volume in the limb are affected in opposite ways. Fig. 1 shows increases of leg volume and changes of blood pressure in response to 0.3, 3.0 and 5.0γ of acetylcholine. The upper tracing is from the leg on the side partially denervated 24 days before the experiment by the removal of 2 ganglia and the crushing of a third in a way that may have injured the contralateral side. Clearly there is no difference in response on the 2 sides in this cat. Operations on 6 out of 10 cats were unsatisfactory for the reasons mentioned. In this group the mean ratio of maximal plethysmograph rise (in mm) on the chronically denervated side to that on the intact or acutely denervated side for 26 intravenous injections of acetylcholine

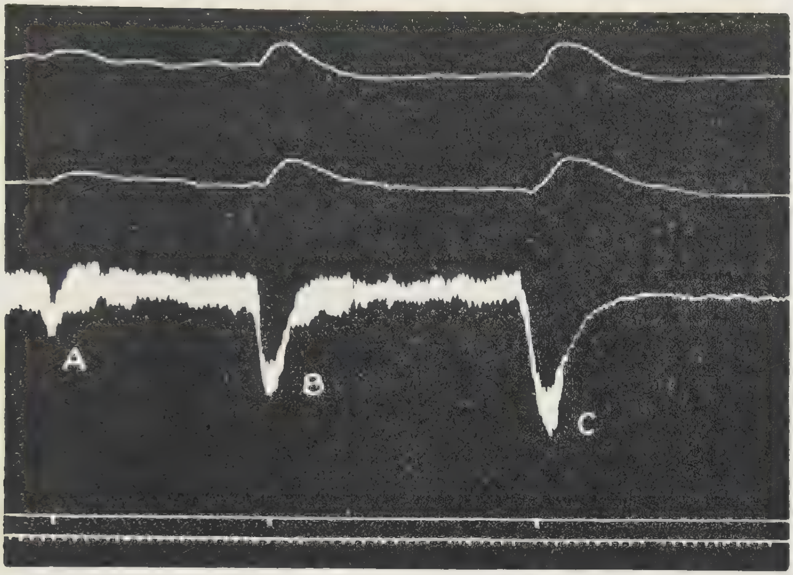

FIG. 1.
Cat in which only part of the sympathetic supply to the right hind limb (upper tracing) had been removed 24 days earlier. A, B and C correspond to injections of 0.3, 3.0 and 5.0 γ acetylcholine, respectively. The responses are the same on the normal and chronically denervated sides. Lower tracing shows blood pressure.

(ranging from 0.1 to 5.0γ) was 0.96, or practically unity, with extremes for the ratio ranging from 2.0 to 0.4.

In 4 of the 10 cats all 4 of the ganglia (L6 and 7, S1 and 2) were removed unilaterally. Fig. 2 shows leg-volume increases and blood-pressure changes in one of these cats in response to doses of 0.3, 0.8, 2.0γ acetylcholine. The lower tracing from the chronically denervated leg clearly reveals a larger rise. One of these 4 cats gave no response at all to acetylcholine on its normal side, while giving typical responses on its denervated side. In the remaining 3 cats, 18 intravenous injections of acetylcholine, ranging from 0.1 to 5.0γ, were made. The mean ratio of the responses on the chronically denervated side to those of the intact or acutely denervated side showed an average sensitization, thus measured, of 230%. Extremes for the ratio for this series ranged from 1.4 to 5.0.

The writer wishes to express his thanks to Dr. W. B. Cannon and Dr. A. Rosenblueth for suggestions and operational aid in connection with this study.

Summary. Sensitization to intravenous injections of acetylcholine by sympathetic denervation of the hind limbs of cats is demonstrated plethysmographically. It is emphasized that care must be

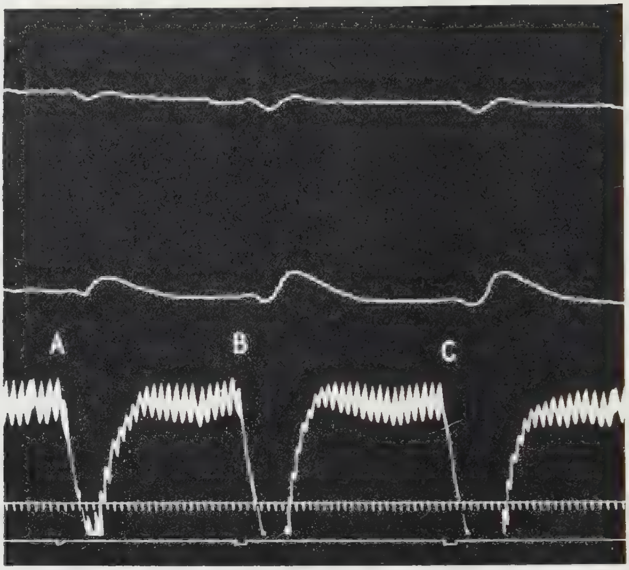

FIG. 2.

Cat in which 4 ganglia, the last 2 lumbar and the first 2 sacral, were removed on the left side (lower tracing) 20 days before the experiment. A, B and C correspond to the injection of 0.3, 0.8 and 2.0 γ acetylcholine, respectively. The chronically denervated side shows a considerably larger response to acetylcholine than does the normal side.

taken to remove completely the sympathetic supply to the limb in question in order to observe sensitization.

13311

A Protein-Free Medium for Primary Isolation of the Gonococcus and Meningococcus.

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It is desired to report the development of a transparent, reproducible medium, containing no heat-labile material such as serum, which is sufficiently stable to withstand autoclave sterilization and storage, and which will support satisfactory primary growth of either meningococci or gonococci. The latter were used as the test

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* Aided by a grant from the Commonwealth Fund.

organisms during the experimental work, since a constant source of primary cultures of the meningococcus is ordinarily not available. The similarity between the two organisms, and the reputedly greater difficulty expected in the cultivation of the gonococcus, indicated that a medium suitable for this organism should prove equally satisfactory for the meningococcus.

As a basis for comparison, the Difco "Proteose No. 3 Chocolate Agar" has been used. This medium has been adopted as a standard in certain laboratories for the diagnosis of gonorrhoea[1] and one of us (J.H.) was thoroughly familiar with its use.

The plan as formulated in beginning the experiments was essentially that followed with considerable success with other organisms, namely, to select the most suitable empirical medium available and attempt to break it down to its essential component parts. The experience of the senior author in 1918 with the Gordon and Hine[2] pea meal extract agar for meningococcus carrier detection had shown that this medium, when carefully prepared, was eminently satisfactory. It proved, upon trial, to be equally so for primary cultivation of the gonococcus, and it was therefore chosen as the starting point in the investigation. It consists essentially of a tryptic digest of meat, agar, and a 5% NaCl extract of pea flour, the latter presumably supplying some essential protein material.

The fractionation of the pea extract proved surprisingly simple, since it could be accomplished by mechanical means. The active portion proved to be, not protein, but starch. This might perhaps have been anticipated on the basis of Vedder's early work.[3] Substitution of ordinary laundry starch completely replaced the growth promoting effect of the pea extract. The effect is evidently due to the starch itself, rather than to some adherent impurity of small molecular size. This has been shown in two ways. First, it was found that a variety of starches,—corn, potato, wheat, rice, arrowroot, etc. were weight for weight identical in their effect. Second, relatively brief hydrolysis of the starch with ptyalin completely destroys the growth-promoting effect. This occurs even before the disappearance of the blue starch-iodine test, and indicates that so-called "soluble starches" are likely to prove unsatisfactory or irregular in their action. The explanation for the effect of the starch is not yet clear. Possibly, as McLeod[4] has suggested in connection with the action of ascitic fluid or serum, it acts as a "protective colloid" against the inhibitory effects of amino acids.

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[1] Cox, D. F., McDermott, M., and Hinton, W. A., *The Gonococcus and Gonococcal Infection,* Science Press, 1939, p. 79-82.

[2] Gordon, M. H., and Hine, T. G. M., *Brit. Med. J.*, 1916, **2**, 678.

[3] Vedder, E. B., *J. Infect. Dis.*, 1915, **16**, 385.

The tryptic digest of meat was next examined. This can be replaced by a rapidly prepared meat infusion together with a complete acid hydrolysate of casein prepared as described elsewhere by Mueller and Johnson[5] and Mueller and Miller.[6] This hydrolysate is now obtainable from the Difco Laboratories, Inc., Detroit, Michigan, under their trade name of "Casamino Acids, Technical." Further examination of the essential components of the meat infusion is now under way. Since it is quite possible that this will prove to be a laborious matter, this report is being made with the hope that the medium in its present form may prove useful to others.

It has been tested in parallel with the Difco chocolate agar in a considerable series of primary cultivations of the gonococcus at the Boston Dispensary. The results have been highly satisfactory. Colonies are frequently larger on the starch medium than on the chocolate. They are easily recognizable, particularly with the aid of the oxidase reagent. There is no appreciably greater tendency for overgrowth of the plates by other organisms, and no significant discrepancies between positives and negatives have been noted. We have recently been fortunate in being able to test the medium on a series of primary isolations of the meningococcus from both spinal fluid and naso-pharyngeal swabs of cases and carriers in Halifax, Nova Scotia. This was made possible through the courtesies of Dr. A. R. Morton, City Health Commissioner and Dr. D. J. MacKenzie, Director of the Provincial Public Health Laboratory. Consistently good results were obtained in connection with this material, and a considerable number of cultures of a Type I meningococcus were readily secured. A correct appraisal of the value of this medium can result only from a more rigorous series of tests in the hands of other workers as well as our own. The details of the preparation follow.

For 1,000 cc Starch Agar. Add 17 g dry shredded agar to 500 cc tap water in a 2 liter flask. Autoclave 15 minutes at 15 pounds to dissolve. While still hot, add the following solution which may be prepared while the agar is being autoclaved:

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[4] McLeod, J. W., Wheatley, M. B., and Phelon, H. V., *Brit. J. Exp. Path.*, 1927, **8**, 25.

[5] Mueller, J. H., and Johnson, E. R., *J. Immunol.*, 1941, **40**, 33.

[6] Mueller, J. H., and Miller, P. A., *J. Immunol.*, 1941, **40**, 21.

Meat infusion*	300	cc
Casein hydrolysate†	17.5	g
Starch paste‡	100	cc
Water	100	”

Adjust pH to 7.4-7.6.

Mix and distribute at once either into test tubes (about 20 cc each for pours, 5 cc for slants) or flasks of 120-200 cc. Autoclave *not more than 10 minutes at 10 pounds.* Over-autoclaving spoils the medium. The flasks can be used to pour plates at once. The tubes may be melted in boiling water and used as needed. The medium seems to give best results when not too moist, either with gonococcus or meningococcus. For either organism a candle jar must be used, and the cultures incubated at about 36° for 24 to 48 hours in the case of gonococcus, or 16 to 24 hours for meningococcus.

13312

Mechanism of Action of Estrogens on Insulin Content of the Rat's Pancreas.*

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The action of estrogens on the pancreas of rats has been demonstrated in a twofold manner. Griffiths, Marks and Young[1, 2] have shown the insulin content of rat pancreas to be increased after implantation of tablets of various estrogens for periods of 2-4 weeks;

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* Meat Infusion—1 pound of meat (chopped lean beef or beef heart), 500 cc water. Suspend meat in water, bring to active boiling, strain through cheese-cloth and filter through paper. For routine use, this infusion may be preserved in the cold room in stoppered bottles containing a few cc of chloroform.

† Casein Hydrolysate—Quantity specified for Difco preparation, Lot S-64123 of "Casamino Acids, Technical."

‡ Starch Paste—Suspend 1.5 g ordinary starch (corn starch or laundry starch, not "soluble starch") in 10 cc cold water. Pour slowly into 90 cc boiling water, while stirring.

* Aided by grants from the Board of Research of the University of California and the Rockefeller Foundation, New York City, and Parke, Davis and Company, Detroit, Michigan. We wish to acknowledge assistance from the Works Projects Administration, Project No. OP-65-1-08, Unit A-5.

[1] Griffiths, M., and Young, F. G., *Nature*, 1940, **146**, 266.

[2] Marks, H. P., and Young, F. G., *Lancet*, December 7, 1940.

Vasquez-Lopez[3] demonstrated marked enlargement of the Golgi apparatus of the islets of Langerhans in similarly treated mice. More recently, the English group of workers[4] also reported an increase in liver weight and glycogen content of such estrinized rats. The authors do not discuss the mechanism of these effects beyond suggesting the possibility that the increase in pancreatic insulin might be secondary to the increase in liver glycogen storage.

In view of the well known overgrowth and cytological changes provoked in the pituitary by the prolonged action of estrogens, it appeared possible that the effect of these compounds on the pancreas might be mediated by the pituitary. This working hypothesis was tested by two approaches. In the first experiment pituitaries of estrogen treated rats were compared with those of untreated controls, regarding their ability to increase upon implantation, the pancreatic insulin content of hypophysectomized rats. In the second experiment, the effect, if any, of estrogens on the pancreatic insulin content of hypophysectomized rats was investigated.

1.[†] Normal rats (donors) were implanted 3 times (once weekly) with 10 mg pellets of alpha estradiol dipropionate. After 18 days' treatment they were sacrificed and their pituitaries as well as those of untreated control rats were immediately implanted into hypophysectomized rats (recipients, each receiving 9 pituitaries in 19 days, with autopsy on the 21st day) and the insulin content of the pancreas of donors as well as of the two types of hypophysectomized recipients compared with their respective controls.[‡]

2. Hypophysectomized rats were implanted 3 times (once week-

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[3] Vasquez-Lopez, E., *Nature*, 1940, **146**, 589.

[4] Griffiths, M., Marks, H. P., and Young, F. G., *Nature*, 1941, **147**, 359.

[†] For a preliminary account of this experiment see 5,6.

[‡] The pancreas of each group of rats were pooled, minced and extracted according to the method of Fisher and Scott.[7] The solutions of crude insulin prepared by this procedure were then assayed by the convulsion method in mice. (For details see [8].) Pancreas extracts which were to be compared were always assayed parallel in groups of 10-15 mice, these assays being repeated from 3 to 6 times. While the insulin content of the pancreas of control groups was found quite variable from one experiment to another (in the earlier experiments possibly owing to an insufficient use of standard insulin solutions), the error inherent in the mouse assay, using 30 to 60 mice per group, was, in many experiments, never found to exceed ±17%. In agreement with others,[2] however, we are not inclined to attribute much significance to differences in the pancreatic insulin of 2 groups of rats of less than 50% unless confirmed by repetition of the experiment. A comparison of the insulin content of normal female and hypophysectomized rats revealed no significant difference (5 groups of the former averaging 0.84 units, and 8 groups of the latter 0.79 units of insulin per 100 g rat).

ly) with alpha estradiol dipropionate—also similar rats receiving at the same time daily injections of a crude pituitary (globulin) fraction. They were autopsied on the 18th day p.o. and of estrogen treatment. The insulin content of the pancreas of these groups was compared with two groups of controls (globulin fraction alone, and untreated).

Results. Experiment 1: The finding of the English investigators[1, 2] has here been clearly confirmed, as shown in the first 2 insulin assays reported on Table I, 1a. Increases in pancreatic insulin of 54% and 90% respectively were obtained in normal rats treated with estrogen pellets.

Comparing the pancreatic insulin content of hypophysectomized rats repeatedly implanted with the pituitaries of estrinized and untreated rats with that of hypophysectomized controls, it was found that the pituitaries of the estrinized rats increased to a moderate extent (39%) the pancreatic insulin, while untreated pituitaries had no 'pancreatrophic' effect (Table I, 1b).

Experiment 2: In hypophysectomized rats no increase in pancreatic insulin content was produced by implants of estrogen pellets. This was true for rats which received the estrogen alone as well as

TABLE I.

Exp. No.	Type of rat	Treatment	No. of rats	Pancreatic insulin	
				U/100 g/rat	Change
1a	Normal ♀, 78-79 days at autopsy	Estrogen pellets*	6	0.8	+54%
		Controls (untreated)	6	0.52	—
	Normal ♀, 84-86 days at autopsy	Estrogen pellets*	6	1.35	+90%
		Controls (untreated)	6	0.70	—
1b	Hypophysectomized ♀, 54-56 days old, 28-30 days p.o. at autopsy	Pituitaries of above *estrinized* rats implanted	6	1.8	+39%
		Pituitaries of above *untreated rats* implanted	6	1.3	0%
		Controls (untreated)	4	1.3	—
2	Hypophysectomized ♀, 72-76 days old, 18 days p.o. at autopsy	Estrogen pellets*	4	0.62	—28%
		Estrogen pellets* and pituitary globulin fraction†	8	0.62	—28%
		Globulin fraction alone†; no implants	9	1.04	+21%
		Controls (untreated)	9	0.86	—
	Normal ♀, 75-84 days at autopsy	Controls	7	0.56	—35%

*10 mg pellets of alpha-estradiol dipropionate (Ciba), implanted once weekly.

†Globulin fraction of beef anterior pituitary extract, containing growth hormone, thyrotrophic hormone, adrenocorticotropic hormone, lactogenic hormone and ICSH (0.2 mg daily intraperitoneally).

TABLE II.
Hypophysectomozed Rats.*
(See Table I, Exp. 2.)

Type of treatment	Body wt (g) Initial‡	Body wt (g) Change	Liver† % of body wt
Estrogen alone	156	—34	3.3
Estrogen + pituitary fraction	149	—15	3.4
Pituitary fraction alone	151	+ 8	3.3
Hypophysectomized controls	142	—16	3.5
Normal controls	156	+26	3.5

*Fasted 18 hours before autopsy.
†All liver glycogens were very low (between 0.03 and 0.05%).
‡On day of hypophysectomy and onset of treatment.

for rats receiving pituitary substitution therapy with the estrogen.§ Both groups contained slightly less insulin in their pancreas (28%) than the untreated hypophysectomized controls (Table I, 2).

Table II summarizes the body weights and liver weights and liver glycogens of these groups. The body weight changes appear of interest in connection with the mechanism of the antagonism of estrogens and "growth"; the liver data in view of Griffiths'[4] finding of an increase in liver weight and glycogen content in normal estrinized rats.

Discussion and Conclusions. The finding of an increase in pancreatic insulin produced by prolonged estrinization of normal rats has been confirmed. It appeared possible that this effect might be mediated by the pituitary which in such estrinized rats is greatly enlarged. A stimulation of production and secretion by the pituitary of greater than usual amounts of a 'pancreatrophic hormone' might account for the observed facts. The pancreatrophic potency of the pituitaries of such estrinized rats was therefore tested, in comparison with that of untreated animals. No pancreatrophic action was evident upon implantation of 9 pituitaries of control rats.‖ The same number of pituitaries of treated rats caused a slight increase in pancreatic insulin content, which by itself, cannot be regarded as sufficient evidence for the increased pancreatrophic potency of the glands. The hypothesis that the effect of the estrogen is mediated by the pituitary is, however, supported by the outcome of the second experiment, *i. e.,* the failure to produce pancreatrophic effects in hypophysectomized rats with the same estrogen treatment which had marked effect in normal rats. That this failure was not due to the pronounced toxic action of the estrogen in such rats was demonstrated by its lack of effect also in rats receiving pituitary substitution therapy besides the implanted estrogen.

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§ The pellets of alpha estradiol dipropionate were not well tolerated by hypophysectomized rats. At autopsy after 18 days' treatment, only 4 of 10 rats were alive, 2 of these moribund. Daily administration of 0.2 mg globulin fraction (prepared from an alkaline extract of beef anterior pituitary) counteracted the toxic effect of the estrogen satisfactorily (8 rats alive and healthy at autopsy). These animals resembled normal rats treated with estrogen pellets.

‖ The lack of pancreatrophic activity in rat pituitaries is attributed not to the absence of the pancreatrophic factor but to a neutralization of its action by another hormone. This will be discussed elsewhere.[8]

It is also noteworthy that estrinization does not lead to increases in liver weights or liver glycogen storage in hypophysectomized rats, as reported for normal rats.[4] It would thus appear very probable that the effect of estrogens on pancreatic insulin content as well as on liver weight and glycogen is mediated by the pituitary gland. Evidence will be discussed elsewhere[8] which indicates that the lactogenic hormone exerts a pancreatic insulin increasing action in normal and hypophysectomized rats. The recent finding[5, 6] that the lactogenic hormone content of and excretion by the pituitary appears to be increased in estrinized rats stands thus in good agreement with the reported observations of an increased 'pancreatrophic' function of the pituitaries of estrinized rats.

Summary. Two types of evidence favor the assumption that the activity of estrogens in elevating the pancreatic insulin content is mediated by the pituitary. (1) The pituitaries of estrinized rats when implanted elevate the pancreatic insulin content of the host animals while the pituitaries of untreated rats do not show this activity. (2) Estrogen which raises pancreatic insulin content in normal rats does not produce this effect in hypophysectomized rats.

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[5] Simpson, M. E., and Evans, H. M., *Anat. Rec.,* 1941, **79**, Suppl. 2.

[6] A detailed account of this work is in preparation for press.

[7] Fisher, A. M., and Scott, D. A., *J. Biol. Chem.,* 1934, **106**, 305.

[8] Fraenkel-Conrat, H. L., Herring, V. V., Simpson, M. E., and Evans, H. M., *Am. J. Physiol.,* in press.

13313

Application of the Technique of Slide Agglutination of Hemolytic Streptococci to Human Sera.*

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This communication is to report that positive results can be obtained by applying the method of slide agglutination to human serum and hemolytic streptococci.

The materials used in the study consisted of strains of hemolytic streptococci and human blood sera. The streptococci were cultured from the nose, throat or suppurative foci of 22 patients ill with streptococcal disease. The blood sera were collected from these same patients for the most part at the end of the third week of their illness.

The cultures used originated from a single colony fished from the original blood agar plate of each patient. The colony was inoculated into rabbit blood broth, and 1 cc of the 18 hour culture was transferred to 5 cc of beef heart infusion broth containing 0.05% dextrose. The resulting 18 hour culture was centrifuged at 2000 rpm for one half hour and the supernatant fluid decanted. If the resulting thick suspension was granular, the culture was transferred to 9 cc of the dextrose broth to which had been added 1 cc of bactotrypsin. The 18 hour growth was likewise treated by centrifuging and decanting and the remaining thick suspension was usually smooth. Typing was carried out by using one loopful of this suspension and one loopful of the rabbit typing serum, diluted to the optimal strength. The 2 were mixed on a slide by tapping the drop 5 to 6 times with the loop and rotating the slide rapidly 5 to 6 times while tilted at an angle of approximately 45 degrees. The organism was considered to be satisfactorily typed only when gross agglutination took place within 5 seconds.

Tests with the patient's sera were done in a manner similar to that used with the rabbit typing sera. Each human sample was tested with the strain of hemolytic streptococci from that patient and with a number of homologous and heterologous strains from other patients. The sera were used both undiluted and serially diluted with

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* Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

TABLE I.
Results of Slide Agglutination of Serum of Patient P.B. (Type 1) and Patient A.P. (Type 3) and Homologous and Heterologous Streptococci.

Strain		Serum PB		Strain		Serum AP	
Patient	Type	0	1-10	Patient	Type	0	1-10
PB	1	+	0	AP	3	+	0
FM	1	+	0	SB	3	+	0
AC	1	+	0	RB	3	+	0
JG	NT*	0	0	JD	3	+	0
BB	NT	0	0	LB	3	+	0
FR	1	+	0	PB	1	0	0
AR	1	+	0	FM	1	0	0
AP	3	0	0	JG	NT	0	0
SB	3	0	0	BB	NT	0	0
RB	3	0	0	PS	1	0	0
PS	1	+	0	PA	1	0	0
PA	1	+	0	FR	1	0	0
JD	3	0	0	AR	1	0	0
LB	3	0	0	AC	1	0	0

*NT = No type obtained with available rabbit sera.

normal saline 1-10, 1-20, 1-40 and in some instances 1-80. No agglutination was found beyond 1-80.

The streptococci were typed, when possible, by the method of slide agglutination as described by Griffith[1] and modified by Boisvert.[2] Type 1 was recovered from 8 patients and Type 3 from 7 patients. Strains from 7 patients could not be typed with the rabbit sera available in our laboratory. Sera from 6 of the 22 patients were found to contain agglutinins for their own strain of streptococci and for homologous strains from other patients, but not for heterologous strains from other patients. Results from 2 of these patients (a Type 1 and a Type 3) are shown in Table I.

Summary. Twenty-two samples of human sera were tested and 6 were positive. They gave agglutination with their own and all homologous strains used. Crossed agglutinations were seen with none, but the element of time in the method employed should be reiterated. The agglutinations were read immediately and by 30 seconds the slides were discarded. The 6 positive sera were from 3 patients with purulent otitis media, 2 of these scarlatinal, 2 patients with empyema, and one patient with paronychia and axillary adenitis.

Conclusions. In 6 of 22 samples of convalescent human sera slide agglutination with the homologous hemolytic streptococci was detectable.

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[1] Griffith, F. J., *J. Hyg.*, Cambridge, England, 1934, **34**, 542.

[2] Boisvert, P. L., *Science*, 1941, **94**, 193.

13314

Stability of Vi Antigen of *Salmonella typhi.**

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The lability of the Vi antigen of the typhoid bacillus has been emphasized as its most marked characteristic. Felix and Pitt[1, 2] found that Vi antigen was destroyed in 90 minutes at 58°C and in an hour at 100°C and disappeared when treated with alcohol, chloroform or toluol. Exposure to ether resulted in a partial loss of the substance. Kauffmann[3] suggested the use of formalized suspensions for agglutination tests. Felix and Bhatnagar[4] reported that formalized suspensions retained their agglutinability by Vi antiserum and their agglutinogenic properties. Serums prepared with formalized suspensions were, however, inferior in protective antibodies to serums prepared with living organisms. They also found that phenolized suspensions progressively lost their agglutinability by Vi antiserum and serums produced with such suspensions contained no Vi agglutinins and were devoid of protective antibodies. Felix and Pitt[5] found that alcohol-treated suspensions retained their agglutinogenic and agglutinin-binding properties but that their agglutinability by Vi serums was "almost annulled or at least very much reduced." Felix and Petrie[6] also stated that alcohol treatment almost annulled the Vi agglutinability. All the workers cited above observed that as the agglutinability of the bacilli by Vi antiserums disappeared they progressively became more agglutinable by O antiserums.

Since the observations of the writer were not in full accord with the descriptions of the characteristics of Vi antigen, particularly as regards its susceptibility to heat and alcohol, a systematic study of

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* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

† Research fellow on grant from the John Simon Guggenheim Memorial Foundation.

[1] Felix, A., and Pitt, R. M., *J. Path. and Bact.*, 1934, **38**, 409.

[2] Felix, A., and Pitt, R. M., *Lancet*, 1934, **2**, 186.

[3] Kauffmann, F., *Z. Hy.*, 1935, **116**, 617.

[4] Felix, A., and Bhatnagar, S. S., *Brit. J. Exp. Path.*, 1935, **16**, 422.

[5] Felix, A., and Pitt, R. M., *Brit. J. Exp. Path.*, 1936, **17**, 81.

[6] Felix, A., and Petrie, G. F., *J. Hyg.*, 1938, **38**, 673.

TABLE I.
Effect of Temperature on Vi Antigen.

	5°C		25°C		37°C		50°C	
	O+ Vi++	O++ Vi—	O+ Vi++	O++ Vi—	O+ Vi++	O++ Vi—	O+ Vi++	O++ Vi—
0.85% saline	1 mo	6 mo	5 da	10 da	4 hr	3 da	20 min	4 hr
Saline + 0.3% formalin	17 da	>7 "	4 "	2 mo	2 "	12 hr	10 "	2 "
Saline + 0.5% phenol	7 "	17 da	2 hr	2 da	20 min	2 "	4 "	15 min
Saline saturated with chloroform	2 hr	7 hr	30 min	5 hr	10 "	30 min	2 "	4 "

influence of temperature and certain chemicals on the substance was undertaken. The Watson strain of *S. typhi* was used because of the constancy of its content of Vi antigen. Additional experiments with other strains of *S. typhi* gave comparable results. In the preparation of Vi suspensions the culture was plated and Vi colonies selected as recommended by Craigie[7] and Giovanardi.[8] This selection was continued until cultures isolated from Vi colonies and maintained in the refrigerator yielded apparently pure Vi growths when transferred. The suspensions used in the tests consisted of organisms scraped from 20 hour agar plate cultures and suspended directly in the vehicle to be tested. All these suspensions were actively agglutinated by pure Vi serum but were not affected by O antiserum. The Vi antiserum used in the tests was prepared with a formalized suspension of a Vi culture of *S. typhi* (Watson). It was freed of H and O agglutinins by absorption with *S. typhi* (H901) before use. In slide agglutination tests the serum had a titer of 1 to 50. It was used in a dilution of 1 to 10. The O serum was prepared with *S. gallinarum* and had a titer of 1 to 150 for the W form of the Watson strain in slide agglutination tests. It was used in a dilution of 1 to 20. While the work reported here is based on slide agglutination tests, these were adequately controlled by expanded tube agglutination tests. The only discrepancy between the slide and tube tests was the absence of O agglutination on the slide with suspensions which in the tube gave traces of O agglutination. These traces of O agglutination occurring in Vi suspensions were commented upon by Felix.[9] The use of the slide test not only allowed the performance of a large number of tests but, in addition, it permitted the immediate determination of the antigenic properties of the suspension, and eliminated further changes during the period of incubation in tube agglutination tests.

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[7] Craigie, J., and Brandon, K. F., *J. Path. and Bact.*, 1936, **43**, 233.

[8] Giovanardi, A., *Zbl. f. Bakt., I, Orig.*, 1938, **141**, 341.

[9] Felix, A., *J. Hyg.*, 1938, **38**, 750.

In Table I are summarized the results of a number of experiments on the thermostability of Vi antigen in saline suspension and in saline suspension with the addition of formalin, phenol and chloroform. Under each temperature are given the average length of time that elapsed before agglutination of the suspensions by O serum appeared (O+,Vi++) and that necessary for the complete disappearance of the agglutinability of the suspensions by Vi antiserum (O++, Vi—). The disappearance of Vi agglutinability is accelerated with increasing temperature. Agglutination by Vi serums no longer occurs after the suspensions are heated at 60°C for 30 minutes or at 100°C for 5 minutes. The addition of formalin to the suspensions hastened the appearance of O agglutinability with a corresponding decrease in the reaction with Vi serum. However, a peculiar characteristic of formalized suspensions is that at low temperatures they retain traces of Vi agglutinability for a longer period than suspensions in saline alone. At higher temperatures this retention of traces of Vi agglutinability is not apparent. The addition of phenol to the suspensions hastens the disappearance of Vi agglutinability and the addition of chloroform has an even more marked effect.

While the results given above agree with the general concept of the lability of Vi antigen, those obtained by treatment with alcohol, glycerin and acetone and by simple dehydration of the bacilli are at complete variance with previous reports. Organisms suspended in glycerin retained their agglutinability by Vi serums and their resistance to O agglutination after 8 months at 25°C. Bacilli suspended in acetone and heated at 56°C for 3 hours, or suspended in absolute alcohol and heated at 75°C for 3 hours, were still strongly agglutinable by Vi serum and inagglutinable by O serum. Bacilli suspended in absolute alcohol or in acetone and dehydrated *in vacuo* over sulfuric acid retained their Vi agglutinability and their O inagglutinability for at least 8 months at 25°C, 2 hours at 100°C and 1 hour at 150°C. Bacilli removed from agar plates and simply dehydrated *in vacuo* behaved in the same way. On the contrary, organisms suspended in 50% alcohol were no longer agglutinable by Vi serum after heating at 50°C for 2 hours. Apparently, resistance of Vi antigen to heat is dependent upon dehydration.

Organisms taken directly from agar plates, suspended in absolute alcohol, heated at 75°C for 3 hours and dehydrated *in vacuo* not only retained their Vi agglutinability, but completely absorbed agglutinins from Vi antiserum prepared with living bacilli. Further, organisms treated in this way upon injection into rabbits gave rise to a serum having a Vi titer of 1 to 2000 in tube agglutination tests. The O and H titers of the serum were each 1 to 1000. Absorption of this serum with living bacilli removed all Vi, H and O agglutinins, thus demonstrating the serological identity of Vi antigen before and after treatment with heat and alcohol. The serum prepared with organisms which had been treated with heat and alcohol was used in mouse protection tests. Its protective power was the same as that of a serum of comparable titer which was prepared with living bacilli.[‡]

The results show that the Vi antigen of the typhoid bacillus is not affected by absolute alcohol and that its resistance to heat is much greater than was previously supposed. This stability to heat and alcohol is also shared by the Vi antigen of *S. paratyphi* C, *S. ballerup* and certain strains of *E. coli* in which Kauffmann[10] described V-W variation. The thermolability described by other investigators is accounted for by the fact that, in previous work, aqueous suspensions were used in the study of Vi antigen.

13315 P

Melanin Formation by Deplanted Fragments of Thalamus in Amphibian Larvae.

Paul Weiss.*

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In the course of experiments on the functional deplantation of brain fragments into the dorsal fin of urodele amphibian larvae described in earlier communications,[1] a striking local pigmentation effect was observed, whenever thalamic grafts were used.

Donors and hosts were larvae of *Amblystoma tigrinum,* between 3 and 4 cm in length. The brain part which produced positive effects is shown as shaded area in Fig. 1. One-half of this portion, either

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[‡] The writer is indebted to Lieut. Geo. F. Luippold of the Army Medical School for performing the mouse protection tests.

[10] Kauffmann, F., *Acta Path. Microb. Scan.*, 1941, **18**, 225.

[*] Aided by the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

[1] Weiss, Paul, Proc. Soc. Exp. Biol. and Med., 1940, **44**, 350; 1941, **46**, 14.

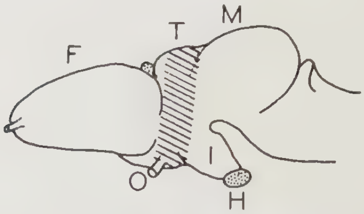


FIG. 1.
Left lateral aspect of the brain of *Amblystoma tigrinum.* F, Forebrain; T, Thalamus; M, Midbrain; I, Infundibulum; H, Hypophysis; O, Optic chiasma.

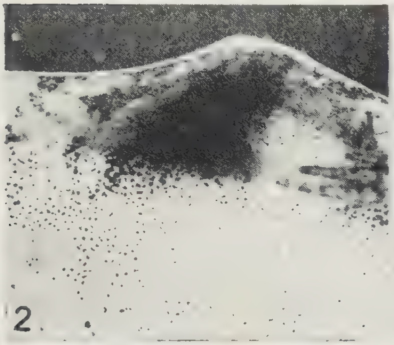
with or without an adjacent slice of midbrain, was inserted into the gelatinous connective tissue of the fin. The grafts were positively free of hypophyseal and adjoining infundibular tissue.

Within a few weeks after the implantation, the vicinity of the grafts turned deep black (Fig. 2), indicating the deposition of vast amounts of newly formed melanin. The host chromatophores were not appreciably affected. Microscopically, the new pigment appears in two forms: (a) as granules inside of richly arborized cells, which give the appearance of hypertrophied chromatophores and which are found both in the interior and along the surface of the grafts; (b) as large compact clumps interspersed among the epidermis cells of the overlying skin (Fig. 4).

Whether the melanotic cells are all of metaplastic thalamic origin, or whether some surrounding host cells have likewise been induced to form pigment, has not yet been determined. However, the fact that deplants into animals which had been deprived of their hypophysis in the embryonic stage and were consequently very lightly pigmented, gave rise to local pigment clusters, points to the graft as the main source of the melanotic cells. Whether the large intercellular pigment clumps of the host epidermis arise as diffuse interstitial pigment deposits, or represent residues of degenerated chromatophores with excessive pigment content, remains to be seen.

Of 8 grafts containing larval thalamic tissue all produced the effect. Of 118 grafts taken from extra-thalamic portions of the central nervous system (forebrain, midbrain, medulla oblongata, spinal cord, spinal ganglia) not a single one has produced any pigmentation (Figs. 3, 5). The effect is, therefore, specific for *thalamus.* It seems to emanate from the thalamic floor, since roof fragments alone fail to produce it.

A continuation of the experiments in *Triturus torosus* larvae has produced similar results, although not wholly comparable because of differences in experimental procedure. In the Triturus series the thalamus was subdivided into 3 portions, (a) roof, (b) an-



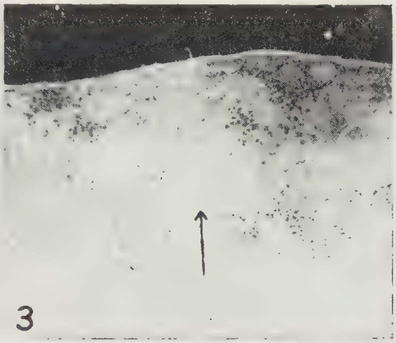
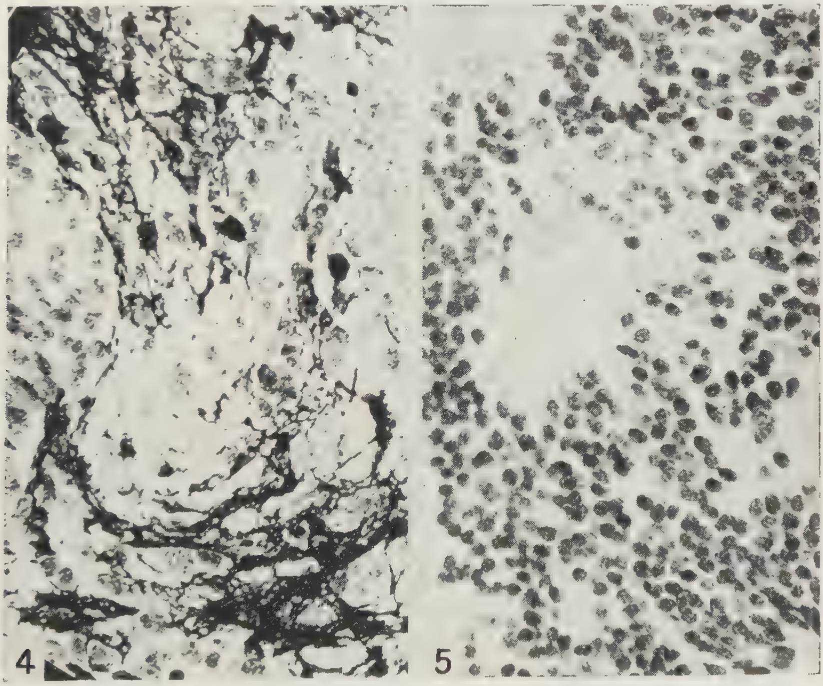


Fig. 2.

Lateral view of portion of dorsal fin containing grafted *thalamus*. Area over graft heavily pigmented. *cca.* 6 ×.

Fig. 3.

Lateral view of dorsal fin containing grafted *midbrain* (light area over arrow). No increase in pigmentation. *cca.* 6 ×.



Figs. 4 and 5.

Sections through a deplant consisting of both thalamus (Fig. 4) and midbrain (Fig. 5), 5 weeks after the operation. 220 ×. Masses of pigmented cells in the thalamic portion; no pigment in the midbrain region.

terior (optic and preoptic) portion of the floor, (c) infundibulum, with or without attached hypophysis. Invariably, heavy pigmentation occurred around (c). In addition, however, both epidermal and dermal host chromatophores within a radius of a few millimeters from the grafts became maximally expanded and finally confluent. This local effect appeared within a week and remained localized. Infundibular fragments without hypophysis have produced it. If pituitary was included, the whole animal, in addition, darkened progressively.

A more detailed analysis, as well as the correlation of the results with those obtained in other forms and with different methods by other workers, is planned for some later date. In conclusion, the experiments indicate triple action on pigment formation by different portions of the diencephalic-hypophyseal complex in urodele amphibians: (1) stimulation of new pigment cell formation and melanin deposition (thalamic floor), (2) local chromatophore expansion (infundibulum), (3) local and diffuse chromatophore expansion (pituitary); (2) and (3) apparently being followed by increase in total melanin content.

13316

A Method for Obtaining Gas Samples of the Total Inspired Air.

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In testing the efficiency of equipment used for administering gases it is important to determine the percentage of the component gases of the total inspired air. A sample of gas may be drawn from the apparatus itself during inspiration at a point near the nose, or alveolar air determinations may be obtained.

Both of these methods have limitations. In a mixture of gases, a sample taken at the beginning or end of inspiration might not be a true index of the percentage of that gas in the total inspired air. An alveolar sample is not an accurate guide of the carbon dioxide concentration in the inspired air, since changes in the volume of ventilation tend to keep the alveolar carbon dioxide concentration constant. Rather wide discrepancies in CO_2 accumulation were found in the inspired air of masks recently tested when the alveolar air samples obtained failed to reveal the significant changes found.[1]

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[1] Barach, Alvan L., and Eckman, Morris, *J. Aviation Med.*, 1941, **12**, 39.

In order to obtain the total inspired air we have developed the following method. This consists of obtaining a sample of gas from the mask, equal in volume and equivalent in rate to a normal inspiration, during the time inspiration would normally be taking place. The samples are collected in a calibrated sampling bottle connected to a reservoir situated at a lower level. By controlling the volume and rate of flow of water from the sampling bottle to the reservoir, samples of gas equivalent to those which would normally be inspired may be obtained.

Description of Apparatus. A sampling bottle (D), calibrated in cc from the mouth down, and a reservoir bottle (E), each of 2 liters capacity are connected by a glass tube of one inch diameter. The bottles are mounted on a wood support frame (C), fitted with a bearing (H). The bearing slips over an axle secured to a large ringstand (F) by a clamp. With this bearing and axle arrangement the support frame may be tilted so that the sampling bottle may be brought above or below the reservoir bottle. A rod (G) fixed to the ringstand near the bottom of the support frame may be swung under the support frame to hold it in any position desired.

Connected to the sampling bottle by means of a rubber stopper is a rubber tube (B), of one-half inch inside diameter, fitted with a pinch clamp. A glass connecting tube (A) is attached to the open end of the rubber tube.

In calibrating the sampling bottle it is important to include the volume of the rubber tube.

Operation. Add about 3 liters of acidified water to the bottles. Tilt the support frame to lower the sampling bottle, open the pinch clamp on the rubber tube and allow all the gas in the bottle, the rubber tube and glass connecting tube to be expelled. Re-clamp the tube.

Raise the sampling bottle by tilting the support frame, until the top of the sampling bottle is well above the water level in the reservoir. The support frame should be fixed in this position by the rod provided for this purpose.

Insert the tip of the glass connecting tube into the mask, or other apparatus to be tested, at a point near the nose. Just as the subject is about to inspire he is told to hold his breath. The pinch clamp is opened long enough to permit a sample equal in volume to the tidal air to be drawn into the sampling bottle.

The size of the sample may be determined from the calibrations on the sampling bottle. By varying the inside diameter of the glass connecting tube or the pitch of the bottles, the speed of intake of

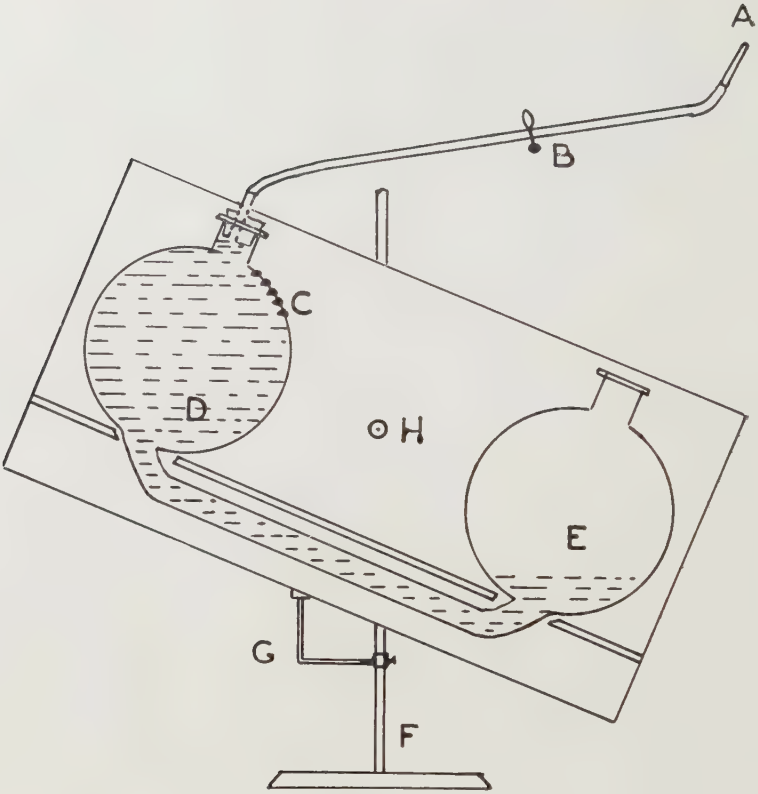

FIG. 1.

the sample may be made to equal the speed of inspiration of the subject.

Conclusions. A method for obtaining an accurate mixed sample of the total inspired air is described. Provision is made for control of the size of the sample and the rate of sampling so that they conform to the inspired air of the subject.

The CO_2 percentage of the inspired air in a mask is more reliably obtained through this method than through alveolar CO_2 samples, since the volume of ventilation tends to keep the CO_2 tension constant. Alveolar samples taken at low barometric pressures, such as 140 mm Hg, are compressed to such a small volume at sea level that analysis may be difficult. Such difficulty is not experienced when taking large inspired air samples for analysis.

13317

Cure of Spectacle Eye Condition in Rats with Biotin Concentrates.*

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The "spectacle eye" condition in rats has been described by several investigators, namely, Goldberger and Lillie,[1] Bourquin and Sherman,[2] Robinson and Newton,[3] Lepkovsky, Jukes and Krause,[4] Sjollema,[5] Karrer, *et al.*,[6] Mackay and Barnes,[7] Oleson, *et al.*,[8, 9] and Pavcek and Baum.[10] During studies on the effect of pantothenic acid on rats reared on various rations it was observed that this typical denudation around the eyes resulted when egg white was added to our basal ration. The eye condition was well defined and this deficiency seemed worthy of further study.

Experimental. The unmodified basal ration had the following percentage composition: sucrose 73, Labco casein 18, salts IV 4, and corn oil 5. Choline hydrochloride was added to this basal at 1 g per kg of ration. The B complex vitamins were fed daily in supplement dishes at the following levels: thiamin hydrochloride 20 γ,

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* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by grants from the Wisconsin Alumni Research Foundation. We are indebted to the Works Progress Administration for assistance in the care of the animals and to Merck and Company, Rahway, New Jersey, for generous supplies of thiamin, riboflavin, nicotinic acid, pyridoxine, pantothenic acid and choline, and Abbott Laboratories, North Chicago, Illinois, for haliver oil.

† Eli Lilly and Company Fellow.

[1] Goldberger, J., and Lillie, R. D., *Pub. Health Rep., U.S.P.H.S.*, 1926, **41**, 1025.

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[4] Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 1936, **115**, 557.

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[6] Karrer, P., Loszt, L., and Verzar, F., *Arch. ges. Physiol.*, 1937, **239**, 644.

[7] Mackay, E. M., and Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 353.

[8] Oleson, J. J., Bird, H. R., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1939, **127**, 23.

[9] Oleson, J. J., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 161.

[10] Pavcek, P. L., and Baum, H. M., *Science*, 1941, **93**, 502.

riboflavin 20 γ, pyridoxine 25 γ and calcium pantothenate 100 γ. Two drops of haliver oil were given each rat per week. The egg white used in these studies will be referred to as "untreated," if the commercial dried egg white was used and "heated," if treated in the following manner: 300 g of dried egg white was stirred in 2 liters of water and the suspension was heated in a steam oven for 18 hours, dried and ground in a mill to a fine powder.

Twenty-one-day-old albino and piebald rats from our colony were used throughout all the experiments. On the above ration the rats grew well and did not develop any symptoms even if left on the ration for 8 weeks. When the casein was completely replaced by "heated" egg white all the animals developed spectacle eye at the end of 5 weeks. On this ration 1 litter of 8 rats developed the eye condition at the end of 3 weeks; however in all the other groups the symptoms were not noticed until the end of 5 weeks.

When 60% of "heated" egg white was incorporated into the ration at the expense of the casein and part of the sucrose, the eye condition did not develop more rapidly. However, a growth variation resulted and it was observed that the animals which grew better exhibited the more severe eye denudation.

In another series 4 animals were placed on the basal containing 18% of "heated" egg white and with the B vitamins 10 mg of inositol (Pfanstiehl) per day was given as suggested by Pavcek and Baum.[10] All animals in this series developed spectacle eye at the end of 5 weeks. Rats showing the syndrome were given 10 mg of inositol per day for 4 weeks and showed no improvement.

Since the heat treatment seemed to be ineffective, in reducing the egg white effect 10% of the casein was replaced by "untreated" egg white. The eye denudation was again produced as quickly and effectively as with the higher levels of "heated" egg white. Four animals were placed on the basal in which 5% of the casein was replaced by "untreated" egg white and at the end of 5 weeks 2 animals showed spectacle eye while at the end of 7 weeks only 1 of the remaining 2 rats showed slight denuding. When 1% of the casein was replaced by "untreated" egg white none of the animals exhibited denuding at the end of 8 weeks.

In order to study the effects of corn oil and pyridoxine in this deficiency a group of rats was placed on a basal diet in which 10% of the casein was replaced by "untreated" egg white. In a group of 4 rats the usual 5% of corn oil was raised to 10% and at the end of 5 weeks complete denuding was again noticed. In another series of 4 animals the pyridoxine was increased to 125 γ per day and no protection was observed. An additional 4 rats were placed on a ration containing 10% of "untreated" egg white, 10% of corn oil and 125 γ of pyridoxine, and no protection was noted. One deficient animal was given 500 γ of pyridoxine per day for 4 weeks and no improvement was observed. Inositol was also inactive when fed in conjunction with 10% "untreated" egg white.

TABLE I.
Spectacle Eye Incidence.

No. of rats	% egg white	Treatment	Modification of ration	Incidence
14	18	Heated		14
10	60	”		10
4	18	”	10 mg Inositol	4
7	10	"Untreated"		7
4	5	”		2
3	1	”		0
4	10	”	10% Corn Oil	4
4	10	”	100 γ Vitamin B_6	4
3	10	”	10% Corn + 100 γ B_6	3
2	10	”	10 mg Inositol	2
3	5	”	2 γ Biotin (orally)	0
3	5	”	2 γ ” (inj.)	0
3	10	”	2 γ ” (orally)	0

Pantothenic acid did not have any protective effect on the development of this syndrome. The egg white content of the diet was varied and 25, 50 and 100 γ levels of pantothenic acid were fed. In a few cases the improvement in growth resulted in more severe denuding, but the onset of the deficiency was not materially shortened.

Many variations in the original basal ration have been studied, namely, replacing the sucrose by lactose, dextrin, starch, cerelose and polished rice, increasing and decreasing the levels of fat and casein but in all cases no spectacle eye was noted. In one series a ration containing cerelose as the carbohydrate and deficient in pantothenic acid showed slight spectacle eye when 3 mg of para-aminobenzoic acid was fed daily over a 7-week period.

Since the presence of egg white in the ration allowed this deficiency to develop it was suggested that biotin might be involved. The rôle of biotin was studied from 3 approaches. In one group of 3 rats showing definite spectacle eye, the egg white was taken out of the ration and cures were noted at the end of 3 weeks. Thus it appeared that the ration contained an appreciable amount of biotin which was unavailable to the animals when egg white was present. We had at our disposal a biotin concentrate (S.M.A. Corporation) No. 5000, and this was injected at a level to supply 2 γ of biotin per day and after 5 days slight pigmentation was noted at the edge of the eyelids. The normal restoration of the hair was complete after 2-3 weeks of treatment. At present 14 rats have been cured in this manner without removing the egg white from the ration. Before we noted that 5% of "untreated" egg white only allowed half of the animals to show spectacle eye, an attempt was made to prevent the development of this symptom. In this group 3 animals were given 2 γ of biotin per day orally and 3 animals were given the same level by injection and at the end of 8 weeks all the group was normal. Since this level of egg white appeared to be minimum for the production of spectacle eye, another group of rats receiving 10% "untreated" egg white was given 2 γ of biotin per day from the start of the experiment and complete protection was observed.

With 5% of the ration as "untreated' egg white the injected animals grew on an average of 13 g more than the ones receiving the same level of biotin orally over a 7-week period. On a ration containing 5% of "untreated" egg white the weight gain of the rats receiving 2 γ of biotin per day over the control group is very significant. For a 7-week period the average weight gain of 4 control animals was 100 g and an average weight gain for 4 animals receiving biotin was 172 g or an increase of 72 g over the control group.

After spectacle eye has developed and if the animals are left on the ration an additional 2-4 weeks there develops a bilateral denudation over the hind quarters and extending up the midline of the back to the head. This further denudation was also noted when 2 γ of biotin was administered in our curative technic with 10% of "untreated" egg white in the ration.

Summary. The spectacle eye syndrome in rats has been produced very consistently by the inclusion of egg white in a synthetic diet which allows good growth in the absence of egg white. Pantothenic acid at levels up to 100 γ per day does not prevent the onset of this condition. Corn oil and pyridoxine or the combination of the two are without effect. Inositol has neither a preventative nor a curative action under these conditions. A biotin concentrate at a level of 2 γ of biotin per day is specific for the prevention and cure of this syndrome. The cure is first noticeable after 5 days and complete restoration of the hair results after 2-3 weeks of treatment. A definite weight gain is noted with rats receiving the biotin concentrates.

13318

The Colorimetric Determination of Cystine in Tobacco Mosaic Virus Protein.

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In a study of the sulfur distribution in tobacco mosaic virus protein, Ross[1] found colorimetric cystine methods, the Sullivan[2] and the Folin-Marenzi[3] as modified by Lugg[4] unsatisfactory in that they gave low and variable results. With the Baernstein[5] method as modified by Kassell and Brand,[6] however, Ross obtained cysteine plus cystine values sufficiently high to account for most of the sulfur of the virus protein.

Since Ross' low and variable results with the colorimetric methods seem to be due to his type of hydrolysis and the use of norite as a decolorizing agent we obtained mosaic tobacco from Dr. D. Breese Jones of the Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture. From this mosaic tobacco the virus protein was prepared by the isolation procedure of Stanley[7] with the trypsin treatment as recommended by Bawden and Pirie.[8] The precipitation, dialysis, concentration, and drying of the virus protein was as done by Ross.[1] The dried samples were kept *in vacuo* over phosphorus pentoxide at room temperature.

Of the dried sample, aliquots (usually 250 mg) were hydrolysed respectively with (A) 2.0 cc of 20% hydrochloric acid for 6 hours; (B) with a mixture of 1.0 cc con. hydrochloric acid and 1.0 cc 95% formic acid for 24 hours;[9] (C) with 6.0 cc of 6N sulfuric acid for 12 hours; (D) with 2.0 cc of 20% hydrochloric acid and 0.1 cc of 20% titanous chloride for 2 hours;[10] (E) with 6.0 cc of 57% hydriodic acid (sp. gr. 1.70) for 18 hours; (F) with 3.0 cc of 57% hydriodic acid and 3.0 cc of 95% formic acid for 24 hours. The hydrolysis with sulfuric acid and with hydriodic acid and the mixture of hydriodic acid and formic acid was done in a nitrogen atmosphere, that is, with nitrogen bubbling through the mixture during hydrolysis. There was considerable humin formation in (A) and (B), little in (C) and none in (D), (E), and (F).

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[1] Ross, A. Frank, *J. Biol. Chem.*, 1940, **136**, 119.

[2] Sullivan, M. X., *Pub. Health Rep., U.S.P.H.S.*, Supplement 78, 1929.

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[4] Lugg, J. W. H., *Biochem. J.*, 1932, **26**, 2160.

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[8] Bawden, F. C., and Pirie, N. W., *Brit. J. Exp. Path.*, 1937, **18**, 275.

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[10] Sullivan, M. X., and Hess, W. C., *J. Biol. Chem.*, 1937, **117**, 423.

(A) The humin in this hydrolysate was practically all in the insoluble form. The mixture was brought to pH 3.5 by 5 N sodium hydroxide added dropwise with stirring and the whole made to 20 cc with 0.1 N hydrochloric acid and filtered. The filtrate was slightly yellow and needed no decolorization.

(B) The hydrolysate was black. After evaporation to a syrup on the water bath it was made up to 20 cc with 0.1 N hydrochloric acid and filtered. The filtrate was a light tan, somewhat darker than (A), but the color was reducible so the filtrate needed no decolorization.

(C) The hydrolysate was neutralized to pH 3.5 and diluted to 25 cc with 0.1 N hydrochloric acid and filtered. The filtrate was golden yellow in color. The organic sulfur was mainly in the form of cystine but part was SH since it gave a good nitroprusside reaction without sodium cyanide. The solution on standing overnight gave no nitroprusside reaction without cyanide and so was determined as cystine.

(D) The hydrolysate was brought to pH 5, approximately, with 5 N sodium hydroxide and centrifuged from the precipitated titanous hydroxide. The precipitate was washed once with 5.0 cc of 0.1 N hydrochloric acid and again centrifuged. The combined solution was slightly yellow and contained only cysteine.

(E) and (F) The hydrolysates were concentrated to a syrup, neutralized with 5 N sodium hydroxide to pH 3.5 and diluted to 25 cc with 0.1 N hydrochloric acid. Both solutions were a light yellow.

The percentage cystine content of the virus protein with different methods of hydrolysis and the use of the Sullivan colorimetric method[2] and the Okuda iodometric method[11] are respectively (A) .47, .48; (B) .53, .48; (C) .67, .71; (D) .64, .64; (E) .73, .70; (F) .69, .69.

Ross' best value by the Baernstein hydriodic acid hydrolysis and iodometric titration was .70% cysteine or cystine when corrected for the recovery of cystine similarly treated.* In the hydriodic acid hydrolysis the cysteine found by the Sullivan method without correction is .73% and by the Okuda method (after oxidizing and reducing with zinc and hydrochloric acid) .70%. The average value by the Sullivan method as determined from the different hydrolysates without humin formation ((C), (D), (E), and (F)) is .68%.

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[11] Okuda, Y., *J. Biochem.*, Japan, 1925, **5**, 217.

* Such a correction, however, assumes that cystine in mixtures behaves like cystine alone.

Summary. The Sullivan colorimetric method can be applied satisfactorily to hydrolysates of tobacco mosaic virus if humin formation is inhibited by hydrolyzing with hydrochloric acid-titanous chloride, or better with hydriodic acid.

13319 P

Inactivity of Nicotinuric Acid in Canine Blacktongue.

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It has been reported[1] on the basis of a single test that nicotinuric acid has a curative action in canine blacktongue, and reference has been made to the "antipellagra value" of nicotinuric acid in a recent review.[2]

We have tested the curative activity of a specimen of nicotinuric acid prepared from glycine ethyl ester and nicotinoyl-chloride by the procedure of Meyer and Graf[3]; melting point 240-41° (Meyer and Graf found 237-8°), neutral equivalent 184 (theoretical 182.7).

The arrangement of the test and basal diet were as previously described.[4] Four dogs were used. With the first 2 dogs curative tests were made by our standard procedure, with daily subcutaneous doses of 6 mg and 8 mg respectively per kg body weight for 5 days. The results were negative. In a third dog given 6 mg per kg a partial cure was obtained, and subsequently a preventive dose of 1 mg per kg daily was given, and the dog declined and died during this treatment. The fourth dog was given 3 mg per kg daily and was completely cured except that its normal weight was not fully restored. The dosing was continued for 60 days, during which time the dog remained healthy. Ten days after the last dose it began to decline rapidly and after 7 days more it died of severe blacktongue. These effects may be contrasted with those due to nicotinic acid itself. In our curative method a daily dose of 1 mg per kg for 5 days produces optimum response; and on basal diets of this type the daily dose needed for prevention of blacktongue is about 0.1 mg per kg.[5]

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[3] Meyer, H., and Graf, R., *Biochem. Z.*, 1930, **229**, 156.

[4] Dann, W. J., Kohn, H. I., and Handler, P., *J. Nutrition*, 1940, **20**, 477.

These results lead us to the conclusion that nicotinuric acid is not a blacktongue-preventive (and therefore not a pellagra-preventive) compound, but is to be grouped with the pyrazine acids,[4] quinolinic acid,[4] 3-amino-pyridine,[6] 2-amino-nicotinic acid,[7] and pyrimidine-4-carboxylic acid.[7] These compounds are not regularly blacktongue-preventive but nevertheless are often able partially to replace nicotinic acid in the diet of the dog, sometimes for a considerable period.

The conclusion that nicotinuric acid is not to be ranked as a true blacktongue-preventive is borne out by the findings of Sarett,[8] who has found that the dog maintained on a low intake of nicotinic acid is unable to hydrolyze nicotinuric acid, but excretes it completely without metabolic change.

This conclusion is of importance in the choice of an assay method for the quantitative estimation of nicotinic acid in biological materials. Snell and Wright[9] have described a convenient and very sensitive microbiological assay, using the organism *Lactobacillus arabinosus* 17-5, and they point out that this organism utilizes nicotinuric acid as efficiently as nicotinic acid. It follows that their method is not suitable when the assay is being performed in order to evaluate the blacktongue- (or pellagra-) preventive activity of any material unless it is free from nicotinuric acid.

Moreover, some of the compounds enumerated above have been listed commercially as pellagra-preventive vitamins. Such a listing is potentially dangerous; nicotinuric acid and the other members of the group to which it belongs should not be classified with nicotinic acid and nicotinamide which can be depended upon to produce rapid clinical improvement in small doses.

Our thanks are due to the John and Mary R. Markle Foundation for the support of this work.

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[5] Birch, T. W., *J. Nutrition*, 1939, **17**, 281.

[6] Subbarow, Y., Dann, W. J., and Meilman, E., *J. Am. Chem. Soc.*, 1938, **60**, 1510; Strong, F. M., Madden, R. J., and Elvehjem, C. A., *J. Am. Chem. Soc.*, 1938, **60**, 2564; Subbarow, Y., and Dann, W. J., *J. Am. Chem. Soc.*, 1938, **60**, 2565.

[7] Handler, P., and Dann, W. J., unpublished observations.

[8] Sarett, H. P., *J. Nutrition*, in press.

[9] Snell, E. E., and Wright, L. D., *J. Biol. Chem.*, 1941, **139**, 675.

13320

Intragastric and Intraintestinal Inoculations with St. Louis Encephalitis Virus.

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While mice can readily be infected with the virus of St. Louis encephalitis by intranasal and intracerebral inoculation, neither subcutaneous, intraperitoneal nor oral administration of the virus ordinarily results in infection. The possible importance of the oral method of infection has again been raised by the work of Harford, Sulkin and Bronfenbrenner.[1] These investigators noted that adult animals which ate infected new born mice not infrequently succumbed to encephalitis.

We have seen occasional instances of infection following oral administration of virus emulsions. This procedure is open to the criticism that in the process of feeding upon the virus emulsion, the animal's nasal mucosa may become contaminated with the virus and hence infection may actually take place through the nasal route.

Direct intragastric and intraintestinal inoculation would effectively obviate the danger of infecting the nasal mucosa during the process of feeding. Brodie[2] reported that he obtained no encephalitic infection either after oral feeding or after intragastric inoculation. Since he carried out gastric injections on only 3 animals, we felt repetition of intragastric and intraintestinal inoculations on a larger scale should be attempted. Each of 28 adult mice was anaesthetized and an opening made into the peritoneal cavity. Under direct observation the stomach was located and .06 cc of a 1/10 dilution of the brain of a mouse killed when moribund from St. Louis encephalitis was injected into the lumen of the stomach with a 26 gauge hypodermic needle. The abdomen was then closed and the animal allowed to recover. Nine animals died within 10 days after inoculation. In each instance the brain, liver, and spleen were removed under sterile conditions, emulsified and inoculated unfiltered intracerebrally into mice. Pieces of stomach and intestines were likewise removed, emulsified in broth and after filtration, inoculated intracerebrally into mice. None of these transplants caused any evidence of encephalitis in the inoculated animals. The cause of death of the animals subjected to intragastric inoculation remains uncertain.

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[1] Harford, C. G., Sulkin, S. E., and Bronfenbrenner, J., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 332.

[2] Brodie, M., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1647.

Eighteen animals survived the intragastric inoculation. Between the nineteenth and thirtieth day after the intragastric inoculation, each of these animals was reinoculated intracerebrally with 100 lethal doses of St. Louis encephalitis virus. Only one animal out of the 18 survived this procedure. Hence, no strong degree of protection developed as a result of the intragastric virus inoculation.

The pooled serum of one group of 5 animals bled 15 days after intragastric inoculation was tested for its protective power against St. Louis encephalitis virus by the virus neutralization technique employed in our laboratory.[3] Protective power against 10 lethal doses of virus was found. This is a rather low titer of antibodies compared to that usually found in convalescent human patients.

Another group of 27 mice were anaesthetized and the abdominal cavity opened. Under direct observation the upper part of the small intestine was located and .06 cc of 1/10 dilution of the brain of a mouse dying of St. Louis encephalitis was injected into the lumen of the intestine. The abdomen was then closed and the animal allowed to recover.

Five animals died within 10 days after operation. The brain, liver, and spleen were removed under sterile conditions, emulsified and inoculated into mice intracerebrally. Pieces of intestine were likewise removed, emulsified in broth and after filtration inoculated intracerebrally into mice.

The transplants from 4 of these animals failed to show any indication of the presence of virus. Presumably death was due to operative trauma. The brain of the fifth animal when emulsified and inoculated intracerebrally into 3 mice gave rise to evidences of encephalitis and caused the death of all 3 animals within 4 to 6 days. The brains of these animals were bacteriologically sterile. Virus neutralization tests carried out, making use of this material showed evidence of the presence of St. Louis encephalitis virus.

Fourteen of the animals which survived the intraintestinal inoculation were reinoculated intracerebrally with 100 lethal doses of St. Louis encephalitis virus between the nineteenth and twenty-third day after the intraintestinal injection. One animal out of 14 survived. This inoculation showed that the vast majority of the mice had developed no immunity as a result of the intraintestinal injection of virus.

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[3] Broun, G. O., and Ruskin, J., *J. Mo. State Med. Assn.*, 1936, **33**, 19.

The pooled serum of one group of animals bled 23 days after intraintestinal inoculation showed no neutralizing antibodies when tested by the virus neutralization test. However, another group of animals bled 21 days after intraintestinal inoculation showed protective power against 200 lethal doses, which is a strong degree of protection. There were 4 animals in this group whose sera were pooled. It was in this group that one animal survived the subsequent intracerebral inoculation of 100 lethal doses of virus.

Summary. Our studies indicate that it is difficult to secure cerebral infection when the virus of St. Louis encephalitis is introduced into the intestinal tract. Nevertheless one definite infection did occur after intraintestinal inoculation. We feel the experimental procedure ruled out the nasal route of infection. Some degree of humoral immunity was produced in mice both by intragastric and intraintestinal inoculation. Only two animals survived the subsequent intracerebral injection of 100 lethal doses of virus, showing that only rarely is adequate protection against infection provided by intragastric or intraintestinal virus inoculations.

13321 P

Influence of Certain Bacteriophages on the H Antigens of *Salmonella poona* and *E. typhi.*

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There is a large body of evidence indicating that the usual site of action on a bacterial cell by a bacteriophage is the soma. However, reports have appeared which call attention to the fact that in the phenomenon of bacteriophagy some other portion of the bacterial cell may be affected. Sertic and Boulgakov[1] described a phage whose action on *E. typhi* was solely on the flagella. Craigie and his associates[2] have shown that in order for certain typhoid phages to manifest themselves Vi antigen must be present in these bacterial cultures. Rakieten *et al.*[3] demonstrated that in the case of mucoid strains of bacteria bacteriophagy mainly affects the capsular material, leaving the somatic portion of the cell relatively unchanged.

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[1] Sertic, V., and Boulgakov, N. A., *Compt. rend. Soc. de Biol.*, 1936, **123**, 887.

[2] Craigie, J., *J. Bact.*, 1936, **31**, 56; Craigie, J., and Brandon, K. F., (a) *Canadian Publ. Health J.*, 1936, **27**, 165; (b) *J. Path. and Bact.*, 1936, **43**, 233; (c) *Ibid.*, 1936, **43**, 249.

We wish to record another instance where the action of 3 different bacteriophages on 2 strains of *Salmonella poona* was limited chiefly to the flagella, the somatic portion of the cell being very slightly, if at all, modified. The strains of *S. poona* were from Dr. Schiff's collection (*S. poona* No. 91, Kauffmann, and *S. poona* No. 733). Both strains develop very well in Savita mediums, macroscopic evidence of growth being visible in broth after 3 hours' incubation at 36°C. Both strains are actively motile, are smooth, and give the usual tests for Salmonella in sugar mediums. Their antigenic pattern is XIII, XXII:z:1,6. The three races of bacteriophage were isolated from sewage. On the basis of their activity toward phage resistant strains (secondary cultures) we have regarded these phages as not being identical. In spite of the fact that these phages contain different types, one of their chief characteristics is their ability to remove the flagella from both strains of *S. poona*.

When 0.25 cc of bacteriophage Ent/5 is added to broth containing 0.1 cc of an 18 hour broth culture of *S. poona* No. 91, no visible evidence of lysis is noted, following incubation and continued for as long as 5 days at 36°C. One can, however, by an appropriate plating method demonstrate plaques. These plaques, in contrast to the well punched out areas usually observed with most phages acting on Salmonella strains, appear as slight depressions in the culture surface, each filled with bacterial growth (secondary culture). On examining broth phage culture mixtures from 3 hours after incubation, the resulting secondary growth exhibits intense Brownian movement, in sharp contrast to the very active, true motility of the control growth. The other 2 phages give a similar reaction on this strain as well as on strain No. 733. When a secondary non-motile culture of strain No. 91 and the original culture are subjected to antigenic analysis the following is found to be the antigenic pattern:

Strain No. 91, smooth, XIII, XXII : z : 1,6 (motile)

Strain No. 91, smooth after being in contact with phage Ent/5 XIII, XXII : — : — (non-motile)

The antigenic analysis was carried out by slide agglutination. One may conclude that the action of the bacteriophage was chiefly on the flagella apparatus. Whether the culture used is in the specific, or non-specific phase, the action of these phages seems to be most evident on the H antigen.

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[3] Rakieten, M. L., Eggerth, A. H., and Rakieten, T. L., *J. Bact.*, 1940, **40**, 529.

A motile strain that is made non-motile through the action of a phage remains non-motile so long as that culture carries that phage, because the phage propagates itself as the bacteria proliferate, and attacks any H antigen that may be formed.

One of us (M.L.R.) has also tested out the pathogenicity of these secondary cultures in mice. It may be reported that these non-motile cultures differ in their pathogenicity. One of the cultures is avirulent, while the other 2 are as virulent as the original smooth strain.

Sixteen other Salmonella strains that are susceptible to several of our phages have been tested, but in no instance have non-motile secondary cultures been obtained. One of us (M.L.R.) has succeeded in isolating 2 phages that attack 2 strains of *E. typhi* in much the same manner as that described above for *Salmonella poona;* both of these recently isolated typhoid strains were rendered non-motile after being in contact with these phages. These studies are being continued.

Conclusions. Three races of bacteriophages have been isolated which attack the H antigens of 2 strains of *Salmonella poona.* Two races of phage have been isolated which are able to attack the H antigen of *E. typhi.* This finding is another example of the selective activity of bacteriophages which is directed particularly against the non-somatic portions of the bacterial cell.

13322 P

Sulfonamide Drugs as Protective Agents Against Carbon Tetrachloride Poisoning.*

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During the last few years we have been interested in the mechanism whereby the subcutaneous injection of various substances such as xanthine, sodium ricinoleate, etc., increases a rat's resistance to the poisonous action of carbon tetrachloride or chloroform. Dur-

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* We gratefully acknowledge our indebtedness to the John and Mary R. Markle Foundation for a grant in aid of this research.

TABLE I.
Protective Action of Sulfonamide Drugs Against Carbon Tetrachloride in Rats.

	Drug used									
Exp. No.	Sulfanilamide		Sulfathiazole		Sulfapyridine		Sulfanilic acid		Controls, no drug	
	lived	died	lived	died	lived	died	lived	died	lived	died
1	6	0	2	0					1	5
2	3	0	3	0					0	4
3	6	0							3	2
4	5	0							2	3
5	6	0							3	3
6	5	0							2	3
7					7	0			1	5
8					4	1	1	4	0	5
9	5	0			5	0	1	4	0	5

ing the course of this investigation, we were led to try the possible effect of oral administration of sulfanilamide on the animal's resistance to carbon tetrachloride. To our considerable surprise, it was found to reduce the mortality rate very markedly. Later experiments confirmed this finding and showed that orally administered sulfanilamide exerted a definite protective action against liver necrosis from acute carbon tetrachloride poisoning.

Experimental. Male and female rats, weighing from 200 to 300 g were used. In the individual experiments animals of the same sex and of approximately the same weight were used for control and treated animals. In the experiments recorded in Table I, the sulfonamide drugs were given by stomach tube in doses of 30 mg per 100 g of body weight. Two doses, one 20 hours, the other 3 hours before the time of acute poisoning, were administered. A few preliminary experiments were carried out with sulfanilamide in doses of 10 and 15 mg per 100 g of body weight. The degree of protection was apparently less than that with the higher doses, consequently the other drugs were not studied at the lower levels. The method of anesthesia was the same as described in a previous article.[1] In the experiments recorded in Table I, mortality alone has been considered as the evidence of protection. Similar experiments, however, have been carried out, involving 18 controls and 22 sulfanilamide-treated animals, in which the duration of anesthesia was reduced so that most of the control animals survived. The remaining animals were sacrificed approximately 36 hours after the time of acute poisoning, their livers removed, sectioned, and examined histologically. Although the degree of protection was not as great as that obtained by the subcutaneous administration of xanthine,

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[1] Forbes, J. C., and Neale, R. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 319.

India ink, etc., it nevertheless was very definite. In some experiments a small amount of overlapping was obtained. At no time, however, has a treated animal been found with definitely greater liver damage than the best control.

Discussion. The mechanism for the protective action of orally administered sulfonamide drugs is unknown. It appears to be in some way related directly to the sulfonamide group since sulfanilic acid exerts very little, if any, protective action.

13323

Prophylactic Effect of Sulfaguanidine Against Ulcerative Cecitis in Rats.

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In the course of nutrition experiments carried out during the past year our results were badly confused by the development in the rat colony of a disorder which we have come to speak of as "cecal disease." Briefly, rats which at birth appear perfectly well begin unaccountably to lose weight. After several weeks one may feel a mass in the abdomen and finally after a further period the animals die. There has been no clinical diarrhea although the feces may be softer than the usual hard pellets. Most of our rats with this disease have been sacrificed when the illness was obvious. The earliest lesion appears on the mesenteric border of the mucosa of the cecum as a tiny, barely visible grey superficial erosion. Later there are clearly evident shallow ulcers covered by adherent filmy greenish yellow membrane. In further stages the ulcers are deeper and there is thickening of the cecal wall so that finally a mass several millimeters thick with deep ragged excavations one to 2 cm in diameter is found. After the earliest stages the cecal contents are usually abnormal; they consist of a thin yellow material which has a characteristic foul odor. A reaction around the cecum soon takes place so that coils of intestine and enlarged lymph nodes become adherent. In association with these masses there are cyst-like pockets of clear fluid. The other viscera as a rule show nothing remarkable except that the spleen may be enlarged. This "cecal disease" has been described by

Buchbinder, Hall, Wilens and Slanetz[1] and more recently, under the title of "Chronic Ulcerative Cecitis," by Stewart and Jones.[2] Their excellent accounts, which include histological and bacteriological studies, agree in all details with our own observations. Buchbinder, Hall, Wilens and Slanetz give evidence that "cecal disease" is caused by strains of paratyphoid bacilli; Stewart and Jones question this position although they agree that paratyphoid bacilli are commonly obtained from the stools of rats with "cecitis." They raise the question of some other primary agent with salmonella as a commensal or secondary invader.

In another paper we shall report on bacteriological studies. Suffice it to say now that *Salmonella enteritidis* may readily be recovered from the stools of rats with "cecitis." Ingestion of one strain caused a violent acute dysentery but so far we have not produced "chronic cecitis," although it seems clear that this disease is an infection of some sort.

The present experiments were carried out for two reasons. First of all there was the purely practical problem of eliminating from our rat colony an epidemic (or endemic) which vitiated all observations connected with the regular work of the laboratory. Secondly we were struck by the fact that chronic cecitis of rats furnishes an admirable disease for chemotherapeutic studies. Unlike acute infections in mice which the animal survives or to which it promptly succumbs, rat cecitis runs a gradual course over months, stool cultures are readily made and in general conditions approximate closely those which obtain in many diseases of man. Finally it was hoped that information as to the value of chemotherapy in the prophylaxis of bowel infection which might be of use in connection with military problems could be obtained.*

Experiments. The general technic of caging, feeding and observing rats in use for many years in this laboratory was followed.[3] Two sets of 21 rats, littermates, taken from "normal" stock were housed in groups of 5 or 6 in adjacent cages. Individual weights varied from 110 to 170 g but the average weights of the animals in the two groups were nearly the same. One group, the control, were placed on the stock laboratory diet consisting of cornmeal, casein, linseed oil cake meal, sardine oil, bone ash and sodium chloride. The other group received the same diet with the addition of sulfaguanidine 0.5%. As rats eat approximately 10-15 g of this stock diet daily each animal ingested about 50-75 mg of the drug daily, a dose on the basis of weight comparable to that tolerated by man. Both sets of animals were weighed at frequent intervals and were observed for signs of diarrhea, abdominal tumor or other disability.

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[1] Buchbinder, L., Hall, L., Wilens, S. L., and Slanetz, C. A., *Am. J. Hyg.*, 1935, **22**, 199.

[2] Stewart, H. L., and Jones, B. F., *Arch. Path.*, 1941, **31**, 37.

* We are indebted to E. R. Squibb and Sons for generous supplies of sulfaguanidine.

[3] MacKay, L. L., and MacKay, E. M., *Am. J. Physiol.*, 1927, **83**, 179.

Inasmuch as over 50% of the stock rats in the laboratory had been developing "cecitis," our purpose was to find out whether sulfaguanidine rations had any prophylactic effect. The experiment extended from Feb. 21, 1941, to May 13, 1941, a total of 82 days. During this time 4 animals of the control group became clinically ill and were sacrificed. Three of them had miscellaneous lesions but no cecitis; these animals were therefore thrown out of the series. The fourth, examined on the 38th day of the experiment, had advanced cecal disease. None of the animals on sulfaguanidine became clinically ill or lost weight. On May 13th both controls and experimental groups were sacrificed under ether anesthesia. At this time all the rats appeared healthy although some of the controls had lost weight and in a few an abdominal mass was palpable.

Results. 1. Effect of Sulfaguanidine on Weight. Fig. 1 shows

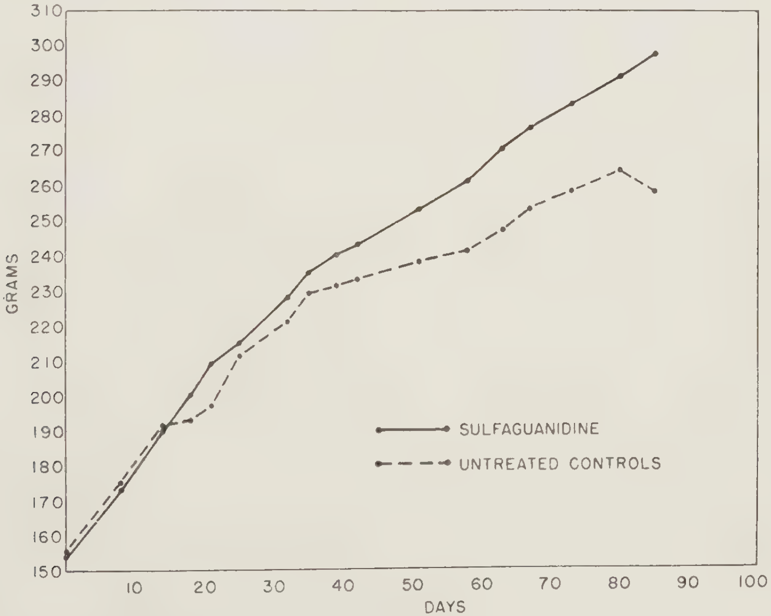

FIG. 1.
Comparison of Weight of Rats with and without Sulfaguanidine in the Diet.

the average weight of the rats with and without sulfaguanidine. It is seen that for the first 3 weeks the curves are almost identical. Thereafter the sulfaguanidine rats gained much faster than the controls which eventually actually lost weight. The lag in the controls seems well explained by the high incidence of "cecitis" as will be shown presently. It is also to be noted that sulfaguanidine did not seem to interfere with the rats' appetite and in general produced no recognizable toxic symptoms. Furthermore, although loss of weight in general was associated with "cecitis" in individual rats there were many discrepancies. Rat No. 32 for example, which at autopsy had a maximal lesion gained 26 g during its last week of life; whereas No. 144 which showed no lesion of any sort gained only 6 g during the same period.

By weighing the feeding boxes each day the approximate amount of food ingested was estimated although there was some error from scattering. The average of a fairly large series of measurements suggests that the controls ate approximately 0.5 g less a day than the sulfaguanidine rats.

2. Incidence and Severity of "Cecitis" in the Two Groups. The cecal lesions at autopsy were graded as follows: 0 = no cecitis, + = superficial erosion not over 0.5 cm in diameter, ++ = shallow ulcer 0.5 to 1 cm in diameter with greenish slough and pericecal adenitis, +++ = ulcer over 1 cm in diameter with thickening of cecal wall and marked pericecitis, ++++ = extreme lesions with huge ulcer and matted adherent coils of intestine.

Table I shows the results. Among the 18 controls 13, or 72%, developed cecitis; in the test group of 21 only 7, or 33.3%, were affected. But the difference in severity of the disease in the two groups is even more impressive. In the test rats such lesions as developed were minimal in all but one case.

Blood Level of Sulfaguanidine. The rats of the test group were exsanguinated at 3 p. m. under ether anesthesia by cutting the aorta, and blood from the entire group was pooled. The blood levels of sulfaguanidine were as follows:†

Duplicate sample No. 1—Free 0.9 mg%; Total 1.1 mg%
" " No. 2— " 1.2 " ; " 1.3 "

Discussion and Summary. Chronic ulcerative cecitis, as it developed spontaneously in a rat colony, presented itself as an ideal experimental disease in which to study the effect of chemotherapy. At the time of the present observations the majority of the animals in

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† We are indebted to Dr. A. P. Richardson for the estimation of blood sulfaguanidine.

TABLE I.
Incidence and Degree of "Cecitis" in Experimental and Control Groups.

Control group		Experimental group	
Rat No.	"Cecitis"	Rat No.	"Cecitis"
144	0	71	0
158	0	81	0
94	0	89	0
32	0	91	0
52	0	93	0
142	+	95	0
30	+	26	0
60	+	31	0
14	++	43	0
50	++	55	0
42	++	61	0
40	+++	41	0
48	+++	49	0
09	+++	51	0
593	+++	04	+
29	+++	47	+
44	++++	79	+
58	++++	06	+
		15	+
		62	+
		83	++

our colony developed lesions of "cecitis" by the time they had reached 300+ g in weight. The addition of 0.5% of sulfaguanidine to the diet produced no deleterious clinical effect nor any visible gross lesions. On the contrary rats on sulfaguanidine gained weight rapidly. The drug even in the small doses used had a striking prophylactic effect against the development of cecitis; not only was the incidence very much lower than in the control group but, even more impressive, such lesions as occurred were minimal in contrast to the advanced disease found in most of the untreated rats. The protective effect of sulfaguanidine is demonstrated further by the failure of the treated animals to lose weight as did the controls with progressive lesions.

The concentration of the drug in the stools was not measured but the relatively low blood levels suggest, as pointed out by Marshall[4] that sulfaguanidine is less readily absorbed than some of the other sulfonamides.

In clinical work on ulcerative colitis, dysentery and other bowel infections in man we have not always been impressed with the therapeutic effects of sulfaguanidine. It is possible, however, that the drug may be of greater value as a prophylactic, and the present experiments suggest that such is the case. Trials in man under proper conditions would seem worth while in connection with military establishments.

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[4] Marshall, E. K., Jr., Bratton, A. Calvin, White, H. J., and Litchfield, J. T., Jr., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 163.

13324

Control of Malaria Infection (*P. lophurae*) in Ducks by Sulfonamides.

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The effect of a number of sulfonamides has been studied in various species of birds infected with different parasites. The course of infection by *Plasmodium cathemerium, P. relictum,* and *P. nucleophilum* in canaries apparently is not significantly altered by sulfonamides.[1, 2] However, sulfapyridine can prevent patent infection by *P. circumflexum* or cause the rapid disappearance of parasites if administered after the infection is patent.[2] No beneficial effects of sulfonamides could be demonstrated in *Hemoproteus columbae* infections in the pigeon.[3] In the Java sparrow infected by *P. praecox,* prontosil was believed effectively to reduce the number of circulating parasites.[4] The action of sulfonamides in infections by *P. lophurae* in the chicken[1] or duck[5] has been reported to be of little or no benefit.

The course of infection by the Coggeshall strain of *P. lophurae* in the duck is particularly suitable for chemotherapeutic study because the number of parasites in the blood usually grows rapidly until death occurs.* In our experiments, Peking ducks 4 or 7 days old were inoculated intravenously with citrated blood from infected

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[1] Coggeshall, L. T., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 768.

[2] Manwell, R. D., Counts, E., and Coulston, F., Proc. Soc. Exp. Biol. and Med., 1941, **46**, 523.

[3] Durand, P., and Villain, *Arch. Inst. Pasteur de Tunis,* 1939, **28**, 94.

[4] Africa, C. M., Dy, F. J., and Soriano, L. J., cited in *Biol. Abstr.,* 1941, **15**, 1455.

[5] Hegner, R., West, E., and Dobler, M., *Am. J. Hyg.,* 1941, **34**, Sec. C, 132.

* We are indebted to Professor Robert Hegner and Miss Evaline West not only for infected ducks carrying the Coggeshall strain of *P. lophuræ* but also for information concerning the transmission and preservation of the parasite in the laboratory.

TABLE I.
Effects of Quinine Hydrochloride, Atabrine Dihydrochloride, or of Two Sulfonamides on Course of Infection by *P. lophuræ* in Ducks.

Group	No. of ducks	Drug*	Total daily dose, g	Avg No. of parasites in 10,000 erythrocytes from day following inoculation Day									
				1	2	3	4	5	6	7	9	11	13
IA	2	——	—	50		585	1265	2970		13900	Dead		
IB	2	Quinine HCl	.015†	46		80	107	67		9	6	4	5
IC	2	Sulfathiazole	.150‡	40		284	497	390		153	40	23	12
ID	2	Sulfadiazine	.150‡	55		275	380	297		157	54	24	8
IIA	2	——	—	198	318		2990		11900		Dead		
IIB	4	Atabrine $(HCl)_2$	.020§	191	158		75		4		0	0	0

*Administered for a six-day period beginning 24 hours after inoculation.
†One dose by stomach tube daily.
‡Estimated from consumption of food containing 0.5% drug.
§Divided into two daily doses by stomach tube.
Average weight of ducks: groups of I, 64 g; groups of II, 114 g.

donors. Ducks of the same age and, as nearly as possible, of the same weight were used. In a given experiment, the doses of infected blood were all obtained from a single donor and after dilution by citrated saline, were injected intravenously in proportion to body weight. Blood for smears was withdrawn at 9 a. m. Tables I and II illustrate some of the variations encountered 24 hours after inoculation. Among 17 control ducks of these and other experiments, in all of which parasitization had been demonstrated, only 3 survived. All the deaths occurred 7-10 days after inoculation.

Knowing that a sulfonamide such as sulfathiazole may have little effect on the course of bacterial infections in mice unless it be administered incorporated in the food by the technic of Bieter and associates[6] we were led similarly to investigate the action of sulfanilamide, sulfathiazole, and sulfadiazine on *P. lophurae* infections in ducks. Moreover, it seemed that investigators of the chemotherapy of bird malaria had neglected the principle of maintaining an adequate blood level of sulfonamide, so important in the control of bacterial infections. An early experiment is summarized in Table I and demonstrates clearly that if sulfathiazole or sulfadiazine is incorporated in the dry poultry mash[†] fed, ducks will survive with a very low parasite count. However, the two sulfonamides appear to be much less effective than the hydrochlorides of either quinine or atabrine.

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[6] Bieter, R. N., Larson, W. P., Levine, M., and Cranston, E. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 202.

[†] New Jersey Poultry Mash, Coöperative G. L. F. Mills, Buffalo.

TABLE II.
Effects of Three Sulfonamides on the Course of Infection by *P. lophuræ* in Ducks.

Group	No. of ducks	Drug*	% fed in dry poultry mash	Mean and S.E. of number of parasites in 10,000 erythrocytes from day following inoculation Day						
				1	3	5	7	9	14	21
IIIA IVA	2 3	Sulfanilamide	1.0	83 ± 20	408 ± 89	651 ± 64	156 ± 29	48 ± 12	272 ± 208	1950 ± 1215†
IIIB IVB	3 3	Sulfathiazole	0.65	91 ± 16	379 ± 57	416 ± 31	145 ± 18‡	61 ± 28	37 ± 22	20 ± 13
IIIC IVC	3 2	Sulfadiazine	0.65	71 ± 21	286 ± 48	278 ± 58	92 ± 16	25 ± 5	16 ± 6	90 ± 60§
IIID IVD	2 2	——	—	92 ± 26	691 ± 126	4680 ± 796	12700 ± 507	Dead‖		

*Administered for a six-day period beginning 24 hours after inoculation.
†Two deaths on 18th and 24th days after inoculation owing to malaria.
‡One death from cardiac puncture 7 days after inoculation.
§One death 16 days after inoculation, cause unknown as smear on 14th day was negative.
‖Controls died 8, 8, 9 and 10 days after inoculation.
Average weight of ducks of all groups: 105 g.

The combined data[‡] of two later experiments are given in Table II and demonstrate how strikingly the parasite count falls to a low level on the 7-9 day although on the ninth day medication had already been stopped for 48 hours and although the severe infection in the controls led to death at about this time (8, 8, 9, and 10 days). In control birds the peak of infection occurred just before death. In birds receiving sulfathiazole or sulfadiazine the peak of infection probably occurred 4-5 days after inoculation or after 3-4 of the 6 days of treatment. In birds eating mash containing 1% sulfanilamide, the course of the infection paralleled that of other treated groups during the first 9 days. However, a marked rise in parasite count appeared later and culminated in the death of 2 of the 5 ducks. In all the birds treated by sulfonamides the parasites were almost entirely gametocytes on the 7-9 days. This fact suggests that these drugs act principally on schizonts. If the parasite count later rose, as in ducks receiving sulfanilamide, schizonts in a normal proportion could then be found. On the fourteenth and twenty-first days no parasites could be found in 2 of the birds receiving sulfadiazine; one of these ducks died from an unknown cause (day 16). One bird, a member of the sulfathiazole group, died with a low parasite count (108) on the seventh day as a result of cardiac puncture.

Each bird was weighed at the time of inoculation, on the third day of treatment and at the end of treatment. Infected birds, whether untreated or receiving sulfathiazole or sulfadiazine gained weight at about the same rate (100-131% increase over starting weight after 7 days). Ducks treated with sulfanilamide gained 41% of their inoculation weight during the same period.

The feeding habits of growing ducks permit only very crude estimates of the daily consumption of the poultry mash. Ducks weighing 60-120 g at inoculation are believed to consume 25-35 g of mash daily.

Specimens for the determination of blood sulfonamide levels[7] were removed at either 8 a. m. or 2 p. m. from all the birds of Table II. We wished to secure data on the height and uniformity of the blood levels and on the degree of conjugation; we believed that diurnal variations such as occur in mice[8] and rats[9] owing to habits of feeding might be found, although the illumination of the ducks was

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[‡] High standard errors are in part the result of combining two experimental groups receiving different infecting doses of parasitized blood.

[7] Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

[8] Litchfield, J. T., Jr., White, H. J., and Marshall, E. K., Jr., *J. Pharm. and Exp. Ther.*, 1939, **67**, 437.

[9] Walker, H. A., and van Dyke, H. B., *J. Pharm. and Exp. Ther.*, 1941, **71**, 138.

TABLE III.
Mean and S.E. of Level of Sulfonamides in Blood of Ducks of Table II.

Drug	% in diet	8 A.M. No. of detms.	8 A.M. Free	8 A.M. Total	2 P.M. No. of detms.	2 P.M. Free	2 P.M. Total
			mg%	mg%		mg%	mg%
Sulfanilamide	1.0	13	20.9 ± 1.6	31.9 ± 1.5	13	19.0 ± 1.9	29.9 ± 2.0
Sulfathiazole	0.65	15	4.9 ± 0.6	5.6 ± 0.7	15	5.2 ± 0.5	6.0 ± 0.5
Sulfadiazine	0.65	14	25.3 ± 1.2	27.6 ± 1.3	13	20.2 ± 1.4	21.9 ± 1.4

quite uniform because of the presence of heating lamps. The blood levels and the degree of conjugation were surprisingly uniform for a given drug (Table III). The average percentages of total drug conjugated were: sulfanilamide, 35%; sulfathiazole, 13%; and sulfadiazine, 8%. The metabolism of the 3 sulfonamides used, so far as this can be inferred from the blood levels, resembles that in mammals. The experiments suggest that in terms of the blood levels of drugs, sulfathiazole is the most effective and sulfanilamide is the least effective.

Summary. Malarial infection *(P. lophurae)* of the young Peking duck usually progresses with rapid multiplication of the parasites in the blood and death after 5-16 days[10] (7-10 days in our series). If sulfonamides are administered by incorporation in the food, the multiplication of parasites is checked after a few days and the birds survive at least several weeks with relatively few or, occasionally, no parasites in the blood (sulfathiazole or sulfadiazine). Sulfanilamide appears to be less effective.

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Comparison Between Destruction of Angiotonin, Hydroxytyramine and Tyramine by Renal Extracts.

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According to Page and Helmer,[1] the arteriolar constriction occurring in experimental renal hypertension is caused by angiotonin, a dialysable substance of unknown chemical constitution. Bing and Zucker,[2, 3, 4] on the other hand, found that experimental hypertension can be caused by pressor amines formed from amino acids through the action of decarboxylating enzymes contained in the renal cortex. Both angiotonin and pressor amines appear to be inactivated by substances contained in the normal kidney.[5, 6, 7] To investigate whether the angiotonin inhibitor was identical with the renal deaminase, which destroys the pressor amines, experiments were performed to ascertain (a) whether both aerobic and anaerobic incubation of angiotonin with renal extracts lead to its destruction; (b) whether fractions of renal extracts which destroy angiotonin also inactivate hydroxytyramine, (an amine formed in the ischemic kidney from the amino acid, *l*-dihydroxyphenylalanine); and (c) whether octyl alcohol, which inhibits deaminization, prevents the destruction of angiotonin by renal extracts.

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[10] Hegner, R., West, E., Ray, M., and Dobler, M., *Am. J. Hyg.*, 1941, **33**, Sec. C, 101.

[1] Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 29.

Methods. Either 0.3-0.5 cc of angiotonin,* 2-3 cc of hydroxytyramine solution,† or 3 cc of 0.1% tyramine solution were added to 2.6-5.0 cc of kidney extract in 500 cc flasks. The volume of fluid was brought to 20 cc with M/15 phosphate buffer (pH 7.4). The flasks were filled with N_2, O_2, or air, and shaken for 3 hours in a constant temperature bath (40°C).‡ The proteins were then precipitated with 5 cc of 50% trichloracetic acid. After filtering, the solutions were extracted 3 times with ether and distilled to dryness *in vacuo.* The residues were redissolved in 6 cc of distilled water and neutralized with 1 N NaOH. To determine their pressor activity, 1-3 cc of the solutions were injected intravenously into cats anesthetized with nembutal (39 mg per kilo of body weight intraperitoneally).

The kidney extract employed in these incubations consisted of either 3-5 cc of a crude renal extract prepared by the method of Bing,[2] or 2.6-5.0 cc (equivalent to 13.5 g of pork kidney) of a more purified extract prepared by the procedure described by Page in which inert proteins were precipitated from a saline extract of pork

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[2] Bing, R. J., *Am. J. Physiol.*, 1941, **132**, 497.

[3] Bing, R. J., and Zucker, M. B., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 343.

[4] Bing, R. J., and Zucker, M. B., *J. Exp. Med.*, 1941, **74**, 235.

[5] Page, I. H., Helmer, O. M., Kohlstaedt, K. G., Fouts, P. J., and Kempf, G. F., *J. Exp. Med.*, 1941, **73**, 7.

[6] Blaschko, H., Richter, D., and Schlossmann, H., *Biochem. J.*, 1937, **31**, 2187.

[7] Holtz, P., and Heise, R., *Arch. Exp. Path. und Pharm.*, 1939, **191**, 87.

* We take this opportunity to thank Dr. I. H. Page for a generous supply of angiotonin.

† Prepared by the anaerobic incubation of 10 mg of *l*-dopa (*l*-dihydroxyphenylalanine, Hoffman-La Roche) with renal cortical extracts.[2]

‡ We wish to express our thanks to Dr. Victor Ross for the use of his apparatus.

TABLE I.
Effect of Kidney Extracts on Angiotonin, Hydroxytyramine and Tyramine.

	Substrate		Kidney extract		Flask filled with	Octyl alcohol	Effect on B.P.
Exp.	Hydroxy-tyramine	Angio-tonin					
	cc	cc	cc			cc	mm Hg
1	1		3	whole kidney	O_2		—20
		.5	3	,, ,,	O_2		0
		.5	3	,, ,,	N_2		—10
		.5			O_2		+35
2		.5	3	,, ,,	N_2		0
		.5	3	,, ,,	O_2		0
3	3		4	cortical	N_2		+30
	3		4	,,	O_2		0
		.5	4	,,	N_2		0
		.5	4	,,	O_2		0
4	3		5	Page's*	O_2		+40
	3		5	,,	N_2		+40
		.5	5	,,	O_2		0
		.5	5	,,	N_2		0
5	2.5		5	,,	O_2		+65
	2.5		5	,,	N_2		+80
		.5	5	,,	O_2		0
		.5	5	,,	N_2		0
6		.3	5	,,	Air		—60
		.3	5	,, (heated)	,,		+30
		.3			,,		+10
7		.3	2.6	,,	,,		—30
		.3			,,		+40
8		.3			,,		+30
		.3			,,	.3	+30
		.3			,,		+30
		.3			,,	.3	+30
9		.3	5	,,	,,		—20
		.3	5	,,	,,	.3	—20
		.3			,,	.3	—15 followed by +30
10		.4	5	,,	,,		0
		.4	5	,,	,,	.2	0
11		.3	2.6	,,	,,		0
		.3	2.6	,,	,,	.3	0
		.3	2.6	,, (heated)	,,		+25
12	2		5	cortical	O_2		0
	2		5	,,	O_2	.3	0
13	2		5	,,	O_2		0
	1.8		5	,,	O_2	.3	0
14	3†		5	,,	O_2		—20
	3†		5	,,	O_2	.3	+60
	3†				O_2		+70
	3†				O_2	.3	+50
15	3†		5	,,	O_2		0
	3†		5	,,	O_2	.3	+90
	3†				O_2		+100
	3†				O_2	.3	+100

*Extract prepared according to method of Page.[5]
†Tyramine, 1 mg per cc.

kidney at pH 4.7.[5] In control experiments the kidney extract was omitted.

Discussion. Experiments in which hydroxytyramine was incubated with crude kidney extracts under aerobic and anaerobic conditions demonstrated that this amine was destroyed only when oxygen was present. Angiotonin, however, lost its pressor effect after both aerobic and anaerobic incubation. Since oxygen is a necessary factor for the action of deaminating enzymes, this result suggests that the principle responsible for the inactivation of angiotonin is not identical with the amine oxydase contained in the kidney.

In 5 instances hydroxytyramine or angiotonin was incubated with a purified renal extract prepared according to the method of Page. Angiotonin was inactivated in every instance, whereas no loss in the pressor effect of hydroxytyramine was noticeable. These results give further proof that the breakdown of angiotonin and that of hydroxytyramine are caused by different factors.

From these experiments it was to be expected that octyl alcohol, which poisons the mono-amine oxydase, would prevent the enzymatic breakdown of hydroxytyramine but would not affect that of angiotonin. In 6 experiments, however, it was shown that both pressor substances were destroyed by kidney extracts even in the presence of the alcohol. On the other hand, tyramine was not destroyed by kidney extract when octyl alcohol was present. Since the work of Blaschko makes it clear that octyl alcohol effectively blocks the oxydative deaminization of both tyramine and hydroxytyramine, these results can only be interpreted by postulating the existence of another aerobic enzyme in kidney extracts capable of destroying hydroxytyramine, but differing from the renal deaminase in its resistance towards octyl alcohol. It is possible that this enzyme is a polyphenolase, since Keilin and Hartree[8] have shown that renal tissue contains an enzyme which oxidizes d- and l-dopa but not d- or l-tyrosine.

Summary. (1) Angiotonin is inactivated following incubation with renal extracts. This destruction does not occur by oxidative deaminization since (a) oxygen is not necessary for the reaction, (b) a fraction of kidney extract can be prepared which destroys angiotonin but fails to inactivate hydroxytyramine, and (c) angiotonin is destroyed in the presence of octyl alcohol. (2) Evidence is presented for the existence of an aerobic polyphenolase in kidney extracts, capable of destroying specific pressor amines.

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[8] Keilin, D., and Hartree, E. F., *Proc. Roy. Soc.*, Series B, 1935-6, **119**, 114.

13326

Inactivation of Vitamin B_1 by Raw Fish.*

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Considerable work in connection with a deficiency disease in foxes occurring as a result of feeding raw fish has been reported in the literature.[1-7] More recent evidence[8, 9] indicates that this deficiency disease is fundamentally a B_1 avitaminosis. Experiments were set up in this laboratory to confirm the above observations and to study in detail the action of fresh fish upon the vitamin B_1 both in a typical fox ration and in pure solutions.

During the course of confirmatory experiments with foxes, it was found possible not only to produce the deficiency by the feeding of raw carp, but also to reproduce the condition in the same animals after recovery from a previous attack. From recent reports in the literature,[8, 9] and also from the results of our own curative therapy, it was possible to postulate that the fundamental condition observed when raw carp was fed was a B_1 avitaminosis. An experiment with chicks was designed in an attempt to confirm this theory. In this study, the basal ration used was identical with that in the fox experiments except that 2-3 drops of Haliver oil were fed to each chick weekly instead of including cod liver oil in the mixed ration. All diets were fed as a wet mash. Fourteen groups of day-old white leghorn chicks (10 per group) were used in these trials. A summary of each group and the results obtained are presented in Table I.

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* Published with the approval of the Director of the Agricultural Experiment Station, University of Wisconsin, Madison, Wis.

Our sincere appreciation is extended to the Wisconsin Conservation Commission for the supply of carp used in these experiments.

The dried brewers' yeast used in these experiments was supplied through the courtesy of Dr. H. A. Levine, Biological Laboratory, Pabst Brewing Company, Milwaukee, Wisconsin.

[1] Green, R. G., *Minn. Wildlife Dis. Invest.*, 1936, **2**, 106.

[2] Green, R. G., *Minn. Wildlife Dis. Invest.*, 1937, **3**, 83.

[3] Green, R. G., *Am. Fur Breeder*, 1938, **11**, 4.

[4] Green, R. G., *Am. Fur Breeder*, 1938, **11**, 6.

[5] Green, R. G., *Am. Fur Breeder*, 1939, **11**, 34.

[6] Green, R. G., and Evans, C. A., *Science*, 1940, **92**, 154.

[7] Coombes, A. I., *Am. Natl. Fur and Market J.*, 1940, **19**, 5.

[8] Green, R. G., Carlson, W. E., and Evans, C. A., *J. Nutrition*, 1941, **21**, 243.

[9] Coombes, A. I., *Am. Natl. Fur and Market J.*, 1941, **20**, 13.

TABLE I.
Effect of Raw Carp on Vitamin B_1 Content of a Typical Fox Ration.

Group No.	Ration	% polyneuritis
1	Basal fox ration	0
2	” + 25% raw whole carp	100
3	” + 25% cooked whole carp	0
4	” + 25% raw carp heads and tails	100
5	” + 25% raw carp muscle meat (skin included)	100
6	” + 25% raw carp entrails	100
7	” + water extract ≎ 25% raw whole carp	0
8	” + ether extract ≎ 25% raw whole carp	40
9	” + residue after water and ether extractions ≎ 25% raw whole carp	100
10	” + 25% raw whole carp + 200 μg B_1/100 g ration	100
11	” + 25% raw whole carp + 350 μg B_1/100 g ration	100
12	” + 25% raw whole carp + 500 μg B_1/100 g ration	80
13	” + 25% whole air-dried carp	50
14	” + 25% canned ocean fish	0

These data indicate that a typical B_1 avitaminosis can be produced in the chick by feeding 25% raw whole carp in a diet that is otherwise adequate in vitamin B_1. The above data also confirm the observations previously made with foxes that the factor (or factors) found in raw whole carp is heat labile, since cooking the fish rendered them safe. In an attempt to detect a specific portion of the fish which would show activity, raw heads and tails, muscle meat (skin included) and entrails were fed to individual groups. All chicks in Group 6 developed polyneuritis at an earlier date than those in Groups 4 and 5.

With the availability of the thiochrome method for the determination of thiamin,[10] experiments were designed (1) to determine whether the actual inactivation of the vitamin B_1 took place in the digestive tract of the animals or within the feed mixture itself, (2) to confirm the observation that cooking renders carp safe, (3) to determine the actual level of inactivation offered by a given amount of carp, and (4) to study in more detail the biochemical phenomena involved.

Since the data obtained from our experiments with the chick (Table I) seemed to indicate that raw entrails were more potent than whole raw carp, the procedure involved the addition of known amounts of crystalline thiamin hydrochloride to a given weight of raw carp entrails. Then, by means of a modification of the thiochrome method, we attempted to recover the added vitamin B_1.

The data in Table II present evidence that the actual inactivation of vitamin B_1 by raw carp takes place within the feed mixture itself.

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[10] Hennessy, D. J., *Ind. and Eng. Chem.* (Anal. Ed.), 1941, **13**, 216.

TABLE II.
In Vitro Studies of Effect of Raw and Cooked Carp Entrails on Crystalline Thiamin Hydrochloride (All determinations made at room temperature).

Wt entrails, g	Incubation time, min	μg vitamin B_1 added	% vitamin B_1 destroyed
2.5 raw	15	100	100
2.5 ”	30	100	100
2.5 ”	60	100	100
2.5 ”	15	200	71
2.5 ”	30	200	90
2.5 ”	60	200	100
2.5 ”	15	300	56
2.5 ”	30	300	62.5
2.5 ”	60	300	95
2.5 ”	720	600	100
2.5 (cooked)	15	100	0
2.5 raw	15	400	45.5
2.5 ”	30	400	50
2.5 ”	60	400	70
2.5 ”	15	500	31.5
2.5 ”	30	500	35.5
2.5 ”	60	500	48
2.5 ”	15	600	25
2.5 ”	30	600	25
2.5 ”	60	600	37
5.0 ”	60	500	100
2.5 (cooked)	120	100	0

These data also confirm the observations previously made *in vivo* that cooking the fish renders them safe. From Table II, there would seem to be a proportional inactivation depending upon the length of time that the fish is in actual contact with the feed mixture. Studies are now in progress to further standardize the conditions involved in the reaction. At the present time, it is impossible to conclude anything definite as to the nature of the reaction. However, from the data obtained, it is possible that the phenomenon is of enzymatic nature.

Summary. Previous observations that a dietary deficiency disease, produced in foxes by feeding raw fish, is fundamentally a B_1 avitaminosis, have been confirmed in the chick. The additional observation has been made that this condition can be reproduced in foxes which have recovered from a previous attack. Evidence obtained from experimentation *in vitro* confirms the observation previously made *in vivo* that the inactivation of vitamin B_1 by raw carp occurs within the feed mixture itself. The level of inactivation depends upon the length of time that the fish is in contact with the feed mixture. Studies to standardize the conditions of the reaction are now in progress. Present evidence indicates that the reaction may be enzymatic in nature.